PCT WORLD INTELLECTUAL PROPERTY ORGANIZATION
International Bureau

INTERNATIONAL APPLICATION PUBLISHED UNDER THE PATENT COOPERATION TREATY (PCT)

(51) International Patent Classification 7 : C12N 15/82, 15/55, 9/18, 5/10, A01H 5/00 | A1

(11) International Publication Number: **WO 00/49164**

(43) International Publication Date: 24 August 2000 (24.08.00)

(21) International Application Number: PCT/US00/03494

(22) International Filing Date: 14 February 2000 (14.02.00)

(30) Priority Data:
09/250,280 16 February 1999 (16.02.99) US

(71) Applicant: SENESCO, INC. [US/US]; 11 Chambers Street, Princeton, NJ 08542 (US).

(72) Inventors: THOMPSON, John, E.; 640 Woolwich Street, Waterloo, Ontario N2J 4G8 (CA). WANG, Tzann-Wei; Apartment 1-1109, 300 Regina Street North, Waterloo, Ontario N2J 3B8 (CA). HUDAK, Katalin; 221-B Taylor Avenue, East Brunswick, NJ 08816 (US). HONG, Yuwen; Building 2, Apartment 810, 300 Regina Street North, Waterloo, Ontario N2J 4H2 (CA).

(74) Agents: DELUCIA, Richard, L. et al.; Kenyon & Kenyon, Suite 700, 1500 K Street, N.W., Washington, DC 20005 (US).

(81) Designated States: AE, AL, AM, AT, AU, AZ, BA, BB, BG, BR, BY, CA, CH, CN, CR, CU, CZ, DE, DK, DM, EE, ES, FI, GB, GD, GE, GH, GM, HR, HU, ID, IL, IN, IS, JP, KE, KG, KP, KR, KZ, LC, LK, LR, LS, LT, LU, LV, MA, MD, MG, MK, MN, MW, MX, NO, NZ, PL, PT, RO, RU, SD, SE, SG, SI, SK, SL, TJ, TM, TR, TT, TZ, UA, UG, UZ, VN, YU, ZA, ZW, ARIPO patent (GH, GM, KE, LS, MW, SD, SL, SZ, TZ, UG, ZW), Eurasian patent (AM, AZ, BY, KG, KZ, MD, RU, TJ, TM), European patent (AT, BE, CH, CY, DE, DK, ES, FI, FR, GB, GR, IE, IT, LU, MC, NL, PT, SE), OAPI patent (BF, BJ, CF, CG, CI, CM, GA, GN, GW, ML, MR, NE, SN, TD, TG).

Published
With international search report.
Before the expiration of the time limit for amending the claims and to be republished in the event of the receipt of amendments.

(54) Title: DNA ENCODING A PLANT LIPASE, TRANSGENIC PLANTS AND A METHOD FOR CONTROLLING SENESCENCE IN PLANTS

(57) Abstract

Regulation of expression of senescence in plants is achieved by integration of a gene or gene fragment encoding senescence-induced lipase into the plant genome in antisense orientation. The carnation gene encoding senescence-induced lipase is identified and the nucleotide sequence is used to modify senescence in transgenic plants.

FOR THE PURPOSES OF INFORMATION ONLY

Codes used to identify States party to the PCT on the front pages of pamphlets publishing international applications under the PCT.

AL	Albania	ES	Spain	LS	Lesotho	SI	Slovenia
AM	Armenia	FI	Finland	LT	Lithuania	SK	Slovakia
AT	Austria	FR	France	LU	Luxembourg	SN	Senegal
AU	Australia	GA	Gabon	LV	Latvia	SZ	Swaziland
AZ	Azerbaijan	GB	United Kingdom	MC	Monaco	TD	Chad
BA	Bosnia and Herzegovina	GE	Georgia	MD	Republic of Moldova	TG	Togo
BB	Barbados	GH	Ghana	MG	Madagascar	TJ	Tajikistan
BE	Belgium	GN	Guinea	MK	The former Yugoslav Republic of Macedonia	TM	Turkmenistan
BF	Burkina Faso	GR	Greece			TR	Turkey
BG	Bulgaria	HU	Hungary	ML	Mali	TT	Trinidad and Tobago
BJ	Benin	IE	Ireland	MN	Mongolia	UA	Ukraine
BR	Brazil	IL	Israel	MR	Mauritania	UG	Uganda
BY	Belarus	IS	Iceland	MW	Malawi	US	United States of America
CA	Canada	IT	Italy	MX	Mexico	UZ	Uzbekistan
CF	Central African Republic	JP	Japan	NE	Niger	VN	Viet Nam
CG	Congo	KE	Kenya	NL	Netherlands	YU	Yugoslavia
CH	Switzerland	KG	Kyrgyzstan	NO	Norway	ZW	Zimbabwe
CI	Côte d'Ivoire	KP	Democratic People's Republic of Korea	NZ	New Zealand		
CM	Cameroon			PL	Poland		
CN	China	KR	Republic of Korea	PT	Portugal		
CU	Cuba	KZ	Kazakstan	RO	Romania		
CZ	Czech Republic	LC	Saint Lucia	RU	Russian Federation		
DE	Germany	LI	Liechtenstein	SD	Sudan		
DK	Denmark	LK	Sri Lanka	SE	Sweden		
EE	Estonia	LR	Liberia	SG	Singapore		

DNA ENCODING A PLANT LIPASE, TRANSGENIC PLANTS AND A METHOD FOR CONTROLLING SENESCENCE IN PLANTS

This application is a continuation-in-part application of application Serial No. 09/105,812, filed June 26, 1998, and incorporated herein in its entirety by reference thereto.

Field of the Invention

The present invention relates to polynucleotides which encode plant polypeptides and which exhibit senescence-induced expression, transgenic plants containing the polynucleotides in antisense orientation and methods for controlling senescence in plants. More particularly, the present invention relates to a plant lipase gene whose expression is induced by the onset of senescence and the use of the lipase gene to control senescence in plants.

Description of the Prior Art

Senescence is the terminal phase of biological development in the life of a plant. It presages death and occurs at various levels of biological organization including the whole plant, organs, flowers and fruit, tissues and individual cells.

Cell membrane deterioration is an early and fundamental feature of senescence. Metabolism of lipids, in particular membrane lipids, is one of several biochemical manifestations of cellular senescence. Rose

petals, for example, sustain an increase in acyl hydrolase activity as senescence progresses that is accompanied by a loss of membrane function (Borochov, et al., Plant Physiol., 1982, 69, 296-299). Cell membrane deterioration is an early and characteristic feature of senescence engendering increased permeability, loss of ionic gradients and decreased function of key membrane proteins such as ion pumps (Brown, et al., Plant Physiol.: A Treatise, Vol. X. Academic Press, 1991, pp.227-275). Much of this decline in membrane structural and functional integrity can be attributed to lipase-mediated phospholipid metabolism. Loss of lipid phosphate has been demonstrated for senescing flower petals, leaves, cotyledons and ripening fruit (Thompson, J.E., Senescence and Aging in Plants, Academic Press, San Diego, 1988, pp. 51-83), and this appears to give rise to major alterations in the molecular organization of the membrane bilayer with advancing senescence that lead to impairment of cell function. In particular, studies with a number of senescing plant tissues have provided evidence for lipid phase separations in membranes that appear to be attributable to an accumulation of lipid metabolites in the membrane bilayer (McKersie and Thompson, 1979, Biochim. Biophys. Acta, 508: 197-212; Chia, et al., 1981, Plant Physiol., 67:415-420). There is growing evidence that much of the metabolism of lipids in senescing tissue is achieved through senescence-specific changes in gene expression (Buchanan-Wollaston, V., J. Exp. Bot., 1997, 307:181-199).

The onset of senescence can be induced by different factors both internal and external. For example, ethylene plays a role in many plants in a variety of plant processes such as seed germination, seedling development, fruit ripening and flower senescence.

Ethylene production in plants can also be associated with trauma induced by mechanical wounding, chemicals, stress (such as produced by temperature and water amount variations), and by disease. Ethylene has been implicated in the regulation of leaf senescence in many plants, but evidence obtained with transgenic plants and ethylene response mutants has indicated that, although ethylene has an effect on senescence, it is not an essential regulator of the process. In many plants ethylene seems to have no role in fruit ripening or senescence. For example in the ripening of fruits of non-climacteric plants such as strawberry, in senescence of some flowers such as day lilies and in leaf senescence in some plants, such as *Arabidopsis*, and in particular, in the monocots there is no requirement for ethylene signaling (Smart, C.M., 1994, New Phytology, 126:419-448; Valpuesta, et al., 1995, Plant Mol. Biol., 28:575-582).

External factors that induce premature initiation of senescence include environmental stresses such as temperature, drought, poor light or nutrient supply, as well as pathogen attack. As in the case of natural (age-related) senescence, environmental stress-induced senescence is characterized by a loss of cellular membrane integrity. Specifically, exposure to environmental stress induces electrolyte leakage reflecting membrane damage (Sharom, et al., 1994, Plant Physiol., 105:305-308; Wright and Simon, 1973, J. Exp. Botany, 24:400-411; Wright, M., 1974, Planta,120:63-69; and Eze et al., 1986, Physiologia Plantarum, 68:323-328), a decline in membrane phospholipid levels (Wright, M., 1974, Planta,120:63-69) and lipid phase transitions (Sharom, et al., 1994, Plant Physiol., 105:305-308), all of which can be attributed to the action of lipase. Plant tissues exposed to environmental stress also

produce ethylene, commonly known as stress ethylene (Buchanan-Wollaston, V., 1997, J. Exp. Botany, 48:181-199; Wright, M., 1974, Planta,120:63-69). As noted above, ethylene is known to cause senescence in some plants.

Membrane deterioration leading to leakage is also a seminal feature of seed aging, and there is evidence that this too refects deesterification of fatty acids from membrane phospholipids (McKersie, B.D., Senarata, T., Walker, M.A., Kendall, E.J. and Hetherington, P.R. In: Senescence and Aging in Plants, Ed. L.D. Nooden and A.C. Leoopold, academic Press, 1988. PP 441-464).

Presently, there is no widely applicable method for controlling onset of senescence caused by either internal or external, e.g., environmental stress, factors. At present, the technology for controlling senescence and increasing the shelf-life of fresh, perishable plant produce, such as fruits, flowers and vegetables relies primarily upon reducing ethylene biosynthesis. For example, U.S. Patent 5,824,875 discloses transgenic geranium plants which exhibit prolonged shelf-life due to reduction in levels of ethylene resulting from the expression of one of three 1-amino-cyclopropane-1-carboxylate (ACC) synthase genes in antisense orientation. Consequently, this technology is applicable to only a limited range of plants that are ethylene-sensitive.

The shelf-life of some fruits is also extended by reducing ethylene biosynthesis, which causes ripening to occur more slowly. Since senescence of these fruits is induced after ripening, the effect of reduced ethylene biosynthesis on shelf-life is indirect. Another approach used to delay fruit ripening is by altering cellular levels of polygalacturonase, a cell-wall

softening enzyme that is synthesized during the early stages of ripening. This approach is similar to controlling ethylene biosynthesis in that it, too, only indirectly affects senescence and again, is only applicable to a narrow range of plants.

Thus, there is a need for a method of controlling senescence in plants which is applicable to a wide variety of plants. It is therefore of interest to develop senescence modulating technologies that are applicable to all types of plants, regardless of ethylene sensitivity.

SUMMARY OF THE INVENTION

This invention is based on the discovery and cloning of a full length cDNA clone encoding a carnation senescence-induced lipase. The nucleotide sequence and corresponding amino acid sequence for the senescence-induced lipase gene are disclosed herein. The nucleotide sequence of the carnation senescence-induced lipase gene has been used as a heterologous probe to detect corresponding genes or RNA transcripts in several plants that are similarly regulated.

The invention provides a method for genetic modification of plants to control the onset of senescence, either age-related senescence or environmental stress-induced senescence. The senescence-induced lipase nucleotide sequences of the invention, fragments thereof, or combinations of such fragments, are introduced into a plant cell in reverse orientation to inhibit expression of the endogenous senescence-induced lipase gene, thereby reducing the level of endogenous senescence-induced lipase and

altering senescence in the transformed plant.

Using the methods of the invention, transgenic plants are generated and monitored for growth and development. Plants or detached parts of plants (e.g., cuttings, flowers, vegetables, fruits, seeds or leaves) exhibiting prolonged life or shelf life with respect to plant growth, flowering, reduced fruit spoilage, reduced seed aging and/or reduced yellowing of leaves due to reduction in the level of senescence-induced lipase are selected as desired products having improved properties including reduced leaf yellowing, reduced petal abscission, reduced fruit spoilage during shipping and storage. These superior plants are propagated. Similarly, plants exhibiting increased resistance to environmental stress, e.g., decreased susceptibility to low temperature (chilling), drought, infection, etc., are selected as superior products.

In one aspect, the present invention is directed to an isolated DNA molecule encoding senescence-induced lipase, wherein the DNA molecule hybridizes with SEQ ID NO:1, or a functional derivative of the isolated DNA molecule which hybridizes with SEQ ID NO:1. In one embodiment of the invention, the isolated DNA molecule has the nucleotide sequence of SEQ ID NO:1, i.e., 100% complementarity (sequence identity) to SEQ ID NO:1. In another embodiment of this aspect of the invention, the isolated DNA molecule contains the nucleotide sequence of SEQ ID NO:4.

In another embodiment of the invention, there is provided an isolated protein encoded by a DNA molecule as described herein above, or a functional derivative thereof. A preferred protein has the amino acid sequence of SEQ ID NO:2, or is a functional derivative

thereof.

Also provided herein is an antisense oligonucleotide or polynucleotide encoding an RNA molecule which is complementary to at least a portion of an RNA transcript of the DNA molecule described herein above, wherein the RNA molecule hybridizes with the RNA transcript such that expression of endogenous senescence-induced lipase is altered. The antisense oligonucleotide or polynucleotide can be full length or preferably has about six to about 100 nucleotides.

The antisense oligonucleotide or polynucleotide is substantially complementary to at least a portion of one strand of a DNA molecule encoding senescence-induced lipase, wherein the DNA molecule encoding senescence-induced lipase hybridizes with SEQ ID NO:1, or is substantially complementary to at least a portion of an RNA sequence encoded by the DNA molecule encoding senescence-induced lipase. In one embodiment of the invention, the antisense oligonucleotide or polynucleotide is substantially complementary to at least a portion of one strand of the nucleotide sequence SEQ ID NO:1 or the RNA transcript encoded by SEQ ID NO:1. In another embodiment, the antisense oligonucleotide is substantially complementary to at least a portion of the 5' non-coding portion of one strand of a DNA molecule encoding senescence-induced lipase, wherein the DNA molecule hybridizes with SEQ ID NO:1. In another embodiment, the antisense oligo- or polynucleotide is substantially complementary to at least a portion of the open reading frame of one strand of the nucleotide sequence SEQ ID NO:4 or the RNA transcript encoded by SEQ ID NO:4.

The invention is further directed to a vector for

transformation of plant cells, comprising

(a) an antisense oligo- or polynucleotide substantially complementary to (1) at least a portion of one strand of a DNA molecule encoding senescence-induced lipase, wherein the DNA molecule encoding senescence-induced lipase hybridizes with SEQ ID NO:1, or (2) at least a portion of an RNA sequence encoded by the DNA molecule encoding senescence-induced lipase; and

(b) regulatory sequences operatively linked to the antisense oligo- or polynucleotide such that the antisense oligo- or polynucleotide is expressed in a plant cell into which it is transformed.

The regulatory sequences include a promoter functional in the transformed plant cell, which promoter may be inducible or constitutive. Optionally, the regulatory sequences include a polyadenylation signal.

The invention also provides a plant cell transformed with the vector as described above, a plantlet or mature plant generated from such a cell, or a plant part of such a plantlet or plant.

The present method is further directed to a method of producing a plant having a reduced level of senescence-induced lipase compared to an unmodified plant, comprising:

(1) transforming a plant with a vector as described above;

(2) allowing the plant to grow to at least a plantlet stage;

(3) assaying the transformed plant or plantlet for altered senescence-induced lipase activity and/or altered senescence and/or altered environmental stress-induced senescence and/or ethylene-induced senescence; and

(4) selecting and growing a plant having altered senescence-induced lipase activity and/or altered senescence and/or altered environmental stressed-induced senescence or ethylene-induced senescence compared to an nom-transformed plant.

A plant produced as above, or progeny, hybrids, clones or plant parts preferably exhibit reduced senescence-induced lipase expression and delayed senescence and/or delayed stress-induced senescence or ethylene-induced senescence.

This invention is further directed to a method of inhibiting expression of endogenous senescence-induced lipase in a plant cell, said method comprising:

(1) integrating into the genome of a plant a vector comprising

(A) an antisense oligo- or polynucleotide complementary to (i) at least a portion of one strand of a DNA molecule encoding endogenous senescence-induced lipase, wherein the DNA molecule encoding the endogenous senescence-induced lipase hybridizes with SEQ ID NO:1, or (ii) at least a portion of an RNA sequence encoded by the endogenous senescence-induced lipase gene; and

(B) regulatory sequences operatively linked to the antisense oligo- or polynucleotide such that the antisense oligo- or polynucleotide is expressed; and

(2) growing said plant, whereby said antisense oligo- or polynucleotide is transcribed and the transcript binds to said endogenous RNA whereby expression of said senescence-induced lipase gene is inhibited.

BRIEF DESCRIPTION OF THE DRAWINGS

Figure 1 depicts the derived amino acid sequence

encoded by the senescence-induced lipase cDNA clone (SEQ ID NO:1) obtained from a carnation flower cDNA library. Consensus motifs within the amino acid sequence are as follows: single underline, amidation site; dotted underline, protein kinase C phosphorylation site; double underline, N-myristoylation site; box border, cAMP phosphorylation site; shadow box, casein kinase II phosphorylation site; cross-hatched box, consensus sequence of lipase family; and dotted box, N-glycosylation site.

Figure 2 depicts the derived full length carnation petal senescence-induced lipase amino acid sequence in alignment with partial sequences of lipase-like proteins. Carlip, full length sequence of carnation petal senescence-induced lipase (SEQ ID NO. 11); arlip, partial sequence of lipase-like protein from *Arabidopsis thaliana* (Gen Bank Accession No. AL021710)(SEQ ID NO. 12); ipolip, partial sequence of a lipase-like sequence from *Ipomea* (Gen Bank Accession No. U55867)(SEQ ID NO. 13); arlipi, partial sequence of lipase-like protein from *Arabidopsis thaliana* (Gen Bank Accession No. U93215)(SEQ ID NO. 14). Identical amino acids among three or four of the sequences are boxed.

Figure 3 shows a Western blot analysis of the fusion protein expression product obtained from carnation lipase cDNA expressed in *E. coli*. The Western blot was probed with antibodies to the senescence-induced lipase protein. Lane 1, maltose binding protein; lane 2, fusion protein consisting of carnation lipase fused through a proteolytic (Factor Xa) cleavage site to maltose binding protein cDNA; lane 3, fusion protein partially cleaved with Factor Xa into free lipase protein (50.2 kDa)and free maltose-binding

protein.

Figure 4 is a Northern blot analysis of RNA isolated from carnation flower petals at different stages of development. Figure 4A is the ethidium bromide stained gel of total RNA. Each lane contained 10 µg RNA. Figure 4B is an autoradiograph of the Northern blot probed with ^{32}P-dCTP-labelled full length carnation senescence-induced lipase cDNA.

Figure 5 is an *in situ* demonstration of lipolytic acyl hydrolase, i.e., lipase activity of the protein product obtained by over expression of the carnation senescence-induced lipase cDNA in *E. coli*. mal, *E. coli* cells containing maltose binding protein alone in a basal salt medium; mLip, *E. coli* cells containing the fusion protein consisting of the carnation senescence-induced lipase fused with maltose binding protein in basal salt medium; 40 mal/40 mLip, *E. coli* cells containing maltose binding protein alone [mal] or the lipase-maltose binding protein fusion product [mLip] in basal salt medium supplemented with Tween 40; 60 mal/60 mLip, *E. coli* cells containing maltose binding protein alone [mal] or the lipase-maltose binding protein fusion product [mLip] in basal salt medium supplemented with Tween 60.

Figure 6A illustrates a restriction enzyme map of the open reading frame of the carnation senescence-induced lipase. The numbers refer to nucleotides in the open reading frame.

Figure 6B is a Southern blot analysis of carnation genomic DNA digested with various restriction enzymes and probed with carnation senescence-induced lipase

cDNA.

Figure 7 is the nucleotide sequence of the carnation senescence-induced lipase cDNA clone. Solid underlining, non-coding sequence of the senescence-induced lipase cDNA; non-underlined sequenced is the open reading frame.

Figure 8 is the amino acid sequence of the carnation senescence-induced lipase cDNA (SEQ ID NO. 2).

Figure 9A is a Northern blot analysis showing the expression of the carnation lipase in stage II petals that have been exposed to 0.5 ppm ethylene for 15 hours. Figure 9A is an ethidium bromide stained gel showing that each of the lanes was loaded with a constant amount of carnation RNA (petals: lanes 1 and 2; leaves: lanes 3 and 4; +, ethylene treated; -, untreated). Figure 9B is an autoradiogram of a Northern blot of the gel in Figure 9A probed with labelled full length carnation petal senescence-induced lipase cDNA.

Figure 10 is a partial nucleotide sequence of tomato leaf genomic senescence-induced lipase (SEQ ID NO. 6) and the corresponding deduced amino acid sequence. The conserved lipase consensus motif is shaded; the sequences of the primers used to generate the genomic fragment are each underlined.

Figure 11 is a bar graph showing the effects of chilling on membrane leakiness. Tomato plants were chilled at 8° for 48 hours and then rewarmed to room temperature. Diffusate leakage (μMhos) from leaf disks was measured for control plants, which had not been chilled, and for chilled plants for 6 and 24 hour periods.

Figure 12 is a Northern blot analysis of tomato leaf RNA isolated from plants that had been chilled at 8°C for 48 hours and rewarmed to ambient temperature for 24 hours. Figure 12A is the ethidium bromide stained gel of total leaf RNA. Figure 12B is an autoradiograph of the Northern blot probed with ^{32}P-dCTP-labelled full length carnation senescence-induced lipase cDNA.

Figure 13 is a partial nucleotide sequence (SEQ ID NO. 15) and corresponding deduced amino acid sequence (SEQ ID NO. 16) of an Arabidopsis EST (GenBank Acc#: N38227) that is 55.5% identical over a 64 amino acid region with the carnation senescence-induced lipase. The conserved lipase consensus motif is shaded.

DETAILED DESCRIPTION OF THE INVENTION

Methods and compositions are provided for altering the expression of senescence-induced lipase gene(s) in plant cells. Alteration of expression of the senescence-induced lipase gene in plants results in delayed onset of senescence and improved resistance to environmental stress, thus extending the plant shelf-life and/or growth period.

A full length cDNA sequence encoding a carnation lipase gene exhibiting senescence-induced expression has been isolated from a cDNA library made from RNA of senescing petals of carnation (*Dianthus caryophyllus*) flowers. Polynucleotide probes corresponding to selected regions of the isolated

carnation flower lipase cDNA sequence as well as the full length carnation lipase cDNA were used to determine the presence of mRNA encoding the lipase gene in senescing carnation leaves, ripening tomato fruit and senescing green bean leaves, as well as environmentally stressed (chilled) tomato leaves. Primers designed from the carnation lipase cDNA were used to generate a polymerase chain reaction (PCR) product using tomato leaf genomic DNA as template. The PCR product contains a partial open reading frame which encodes a partial protein sequence including the conserved lipase consensus motif, ITFTGHSLGA (SEQ ID NO:3). The tomato nucleotide sequence has 53.4% sequence identity with the carnation senescence-induced lipase sequence and 43.5% identity with *Arabidopsis* lipase sequence. The *Arabidopsis* lipase sequence has 44.3% identity with the carnation nucleotide sequence.

The senescence-induced lipase gene of the present invention was isolated by screening a cDNA expression library prepared from senescing carnation petals with antibodies raised against cytosolic lipid-protein particles, a source of the carnation lipase. A positive full-length cDNA clone corresponding to the senescence-induced lipase gene was obtained and sequenced. The nucleotide sequence of the senescence-induced lipase cDNA clone is shown in SEQ ID NO:1. The cDNA clone encodes a 447 amino acid polypeptide (SEQ ID NO: 2) having a calculated molecular mass of 50.2 kDa. Expression of the cDNA clone in *E. coli* yielded a protein of the expected molecular weight that exhibits acyl hydrolase activity, i.e., the expressed protein hydrolyzes *p*-nitrophenylpalmitate, phospholipid and triacylglycerol. Based on the expression pattern of the

enzyme in developing carnation flowers and the activity of the protein, it is involved in senescence.

Northern blots of carnation petal total RNA probed with the full length carnation cDNA show that the expression of the senescence-induced lipase gene is significantly induced just prior to the onset of senescence (Figure 4). Northern blot analyses also demonstrate that the senescence-induced lipase gene is induced by environmental stress conditions, e.g., chilling (Figure 12) and ethylene (Figures 4 and 9), which is known to be produced in response to environmental stress. The Northern blot analyses show that the presence of carnation senescence-induced lipase mRNA is significantly higher in senescing (developmental stage IV) than in young stage I, II and III carnation petals. Furthermore, ethylene-stimulated stage II flowers also show higher senescence-induced lipase gene expression. Similarly, plants that have been exposed to chilling temperatures and returned to ambient temperature also show induced expression of the senescence-induced lipase gene coincident with the development of chilling injury symptoms (e.g., leakiness) (Figures 11 and 12). The overall pattern of gene expression in various plants, e.g., carnation, green beans, tomato, and various plant tissues, e.g., leaves, fruit and flowers, demonstrates that the lipase gene of the invention is involved in the initiation of senescence in these plants and plant tissues. Thus, it is expected that by substantially repressing or altering the expression of the senescence-induced lipase gene in plant tissues, deterioration and spoilage can be delayed, increasing the shelf-life of perishable fruits, flowers and vegetables. This can be achieved by producing transgenic plants in which the lipase cDNA or an oligonucleotide fragment thereof is expressed in the

antisense configuration in fruits, flowers and vegetables , preferably using a constitutive promoter such as the CaMV 35S promoter, or using a tissue-specific or senescence-inducible promoter.

The carnation senescence-induced lipase gene is a single copy gene. Southern blot analysis of carnation genomic DNA cut with various restriction enzymes that do not recognize sequences within the open reading frame of the senescence-induced lipase cDNA was carried out. The restriction enzyme-digested genomic DNA was probed with ^{32}P-dCTP-labelled full length cDNA (SEQ ID NO:1). Under high stringency hybridization conditions, only one restriction fragment hybridizes to the cDNA clone (68°C for both hybridization and washing; washing buffer :0.2% x SSC, 0.1% SDS). Thus, the carnation senescence-induced lipase gene is a single copy gene (Figure 6). The fact that this gene is not a member of a multigene family in carnations strongly suggests that it is a single copy gene in other plants. Thus, knowledge of the complete nucleotide sequence of the carnation senescence-induced lipase gene is sufficient for the isolation of the senescence-induced lipase gene from various other plant species. Indeed, as demonstrated herein, oligonucleotide primers based on the carnation cDNA sequence have been successfully used to generate tomato leaf senescence-induced lipase gene fragments by polymerase chain reactions using tomato leaf genomic DNA as template.

The cloned senescence-induced lipase gene or fragment(s) thereof, when introduced in reverse orientation (antisense) under control of a constitutive promoter, such as the fig wart mosaic virus 35S promoter, the cauliflower mosaic virus promoter CaMV35S or the MAS promoter, can be used to genetically modify

plants and alter senescence in the modified plants. Selected antisense sequences from other plants which share sufficient sequence identity with the carnation senescence-induced lipase gene can be used to achieve similar genetic modification. One result of the genetic modification is a reduction in the amount of endogenous translatable senescence-induced lipase-encoding mRNA. Consequently, the amount of senescence-induced lipase produced in the plant cells is reduced, thereby reducing the amount of cell membrane damage and cell leakage, e.g., reduced leaf, fruit and/or flower spoilage, due to aging or environmental stress. The genetic modification can effect a permanent change in the senescence-induced lipase levels in the plant and be propagated in offspring plants by selfing or other reproductive schemes. The genetically altered plant is used to produce a new variety or line of plants wherein the alteration is stably transmitted from generation to generation. The present invention provides for the first time the appropriate DNA sequences which may be used to achieve a stable genetic modification of senescence in a wide range of different plants.

For the identification and isolation of the senescence-induced lipase gene, in general, preparation of plasmid DNA, restriction enzyme digestion, agarose gel electrophoresis of DNA, polyacrylamide gel electrophoresis of protein, Southern blots, Northern blots, DNA ligation and bacterial transformation were carried out using conventional methods well-known in the art. See, for example, Sambrook, J. et al., Molecular Cloning: A Laboratory Manual, 2nd ed., Cold Spring Harbor Press, Cold Spring Harbor, NY, 1989. Techniques of nucleic acid hybridization are disclosed by Sambrook (Supra).

As used herein, the term "plant" refers to either a whole plant, a plant part, a plant cell or a group of plant cells. The type of plant which can be used in the method of the invention is not limited and includes, for example, ethylene-sensitive and ethylene-insensitive plants; fruit bearing plants such as apricots, apples, oranges, bananas, grapefruit,pears, tomatoes, strawberries, avocados, etc.; vegetables such as carrots, peas, lettuce, cabbage, turnips, potatoes, broccoli, asparagus, etc.; flowers such as carnations, roses, mums, etc.; and in general, any plant that can take up and express the DNA molecules of the present invention. It may include plants of a variety of ploidy levels, including haploid, diploid, tetraploid and polyploid.

A transgenic plant is defined herein as a plant which is genetically modified in some way, including but not limited to a plant which has incorporated heterologous or homologous senescence-induced lipase DNA or modified DNA or some portion of heterologous senescence-induced lipase DNA or homologous senescence-induced lipase DNA into its genome. The altered genetic material may encode a protein, comprise a regulatory or control sequence, or may be or include an antisense sequence or encode an antisense RNA which is antisense to the endogenous senescence-induced lipase DNA or mRNA sequence or portion thereof of the plant. A "transgene" or "transgenic sequence" is defined as a foreign gene or partial sequence which has been incorporated into a transgenic plant.

The term "hybridization" as used herein is generally used to mean hybridization of nucleic acids at appropriate conditions of stringency as would be readily

evident to those skilled in the art depending upon the nature of the probe sequence and target sequences. Conditions of hybridization and washing are well known in the art, and the adjustment of conditions depending upon the desired stringency by varying incubation time, temperature and/or ionic strength of the solution are readily accomplished. See, for example, Sambrook, J. et al., Molecular Cloning: A Laboratory Manual, 2nd edition, Cold spring harbor Press, Cold Spring harbor, New York, 1989. The choice of conditions is dictated by the length of the sequences being hybridized, in particular, the length of the probe sequence, the relative G-C content of the nucleic acids and the amount of mismatches to be permitted. Low stringency conditions are preferred when partial hybridization between strands that have lesser degrees of complementarity is desired. When perfect or near perfect complementarity is desired, high stringency conditions are preferred. For typical high stringency conditions, the hybridization solution contains 6x S.S.C., 0.01 M EDTA, 1x Denhardt's solution and 0.5% SDS. Hybridization is carried out at about 68°C for about 3 to 4 hours for fragments of cloned DNA and for about 12 to about 16 hours for total eukaryotic DNA. For lower stringencies the temperature of hybridization is reduced to about 12°C below the melting temperature (T_M) of the duplex. The T_M is known to be a function of the G-C content and duplex length as well as the ionic strength of the solution.

As used herein, the term "substantial sequence identity" or "substantial homology" is used to indicate that a nucleotide sequence or an amino acid sequence exhibits substantial structural or functional equivalence with another nucleotide or amino acid sequence. Any structural or functional differences

between sequences having substantial sequence identity or substantial homology will be *de minimis*; that is, they will not affect the ability of the sequence to function as indicated in the desired application. Differences may be due to inherent variations in codon usage among different species, for example. Structural differences are considered *de minimis* if there is a significant amount of sequence overlap or similarity between two or more different sequences or if the different sequences exhibit similar physical characteristics even if the sequences differ in length or structure. Such characteristics include for example, ability to hybridize under defined conditions, or in the case of proteins, immunological crossreactivity, similar enzymatic activity, etc.

Additionally, two nucleotide sequences are "substantially complementary" if the sequences have at least about 70 percent, more preferably, 80 percent and most preferably about 90 percent sequence similarity between them. Two amino acid sequences are substantially homologous if they have at least 50%, preferably 70% similarity between the active portions of the polypeptides.

The term "functional derivative" of a nucleic acid (or poly- or oligonucleotide) is used herein to mean a fragment, variant, homolog, or analog of the gene or nucleotide sequence encoding senescence-induced lipase. A functional derivative may retain at least a portion of the function of the senescence-induced lipase encoding DNA which permits its utility in accordance with the invention. Such function may include the ability to hybridize with native carnation senescence-induced lipase or substantially homologous DNA from another plant which encodes senescence-induced lipase or with an

mRNA transcript thereof, or, in antisense orientation, to inhibit the transcription and/or translation of plant senescence-induced lipase mRNA, or the like.

A "fragment" of the gene or DNA sequence refers to any subset of the molecule, e.g., a shorter polynucleotide or oligonucleotide. A "variant" refers to a molecule substantially similar to either the entire gene or a fragment thereof, such as a nucleotide substitution variant having one or more substituted nucleotides, but which maintains the ability to hybridize with the particular gene or to encode mRNA transcript which hybridizes with the native DNA. A "homolog" refers to a fragment or variant sequence from a different plant genus or species. An "analog" refers to a non-natural molecule substantially similar to or functioning in relation to either the entire molecule, a variant or a fragment thereof.

By "altered expression" or "modified expression" of a gene, e.g., the senescence-induced lipase gene, is meant any process or result whereby the normal expression of the gene, for example, that expression occurring in an unmodified carnation or other plant, is changed in some way. As intended herein, alteration in gene expression is complete or partial reduction in the expression of the senescence-induced lipase gene, but may also include a change in the timing of expression, or another state wherein the expression of the senescence-induced lipase gene differs from that which would be most likely to occur naturally in an unmodified plant or cultivar. A preferred alteration is one which results in reduction of senescence-induced lipase production by the plant compared to production in an unmodified plant.

In producing a genetically altered plant in accordance with this invention, it is preferred to select individual plantlets or plants by the desired trait, generally reduced senescence-induced lipase expression or production. Expression of senescence-induced lipase can be quantitated, for example in a conventional immunoassay method using a specific antibody as described herein. Also, senescence-induced lipase enzymatic activity can be measured using biochemical methods as described herein.

In order for a newly inserted gene or DNA sequence to be expressed, resulting in production of the protein which it encodes, or in the case of antisense DNA, to be transcribed, resulting in an antisense RNA molecule, the proper regulatory elements should be present in proper location and orientation with respect to the gene or DNA sequence. The regulatory regions may include a promoter, a 5'-non-translated leader sequence and a 3'-polyadenylation sequence as well as enhancers and other regulatory sequences.

Promoter regulatory elements that are useful in combination with the senescence-induced lipase gene to generate sense or antisense transcripts of the gene include any plant promoter in general, and more particularly, a constitutive promoter such as the fig wart mosaic virus 35S promoter, the cauliflower mosaic virus promoter, CaMV35S promoter, or the MAS promoter, or a tissue-specific or senescence-induced promoter, such as the carnation petal GST1 promoter or the *Arabidopsis* SAG12 promoter (See, for example, J.C. Palaqui et al., Plant Physiol., 112:1447-1456 (1996); Morton et al., Molecular Breeding, 1:123-132 (1995); Fobert et al., Plant Journal, 6:567-577 (1994); and Gan et al., Plant Physiol., 113:313 (1997), incorporated

herein by reference). Preferably, the promoter used in the present invention is a constitutive promoter.

Expression levels from a promoter which is useful for the present invention can be tested using conventional expression systems, for example by measuring levels of a reporter gene product, e.g., protein or mRNA in extracts of the leaves, flowers, fruit or other tissues of a transgenic plant into which the promoter/reporter have been introduced.

The present invention provides antisense oligonucleotides and polynucleotides complementary to the gene encoding carnation senescence-induced lipase or complementary to a gene or gene fragment from another plant, which hybridizes with the carnation senescence-induced lipase gene under low to high stringency conditions. Such antisense oligonucleotides should be at least about six nucleotides in length to provide minimal specificity of hybridization and may be complementary to one strand of DNA or mRNA encoding senescence-induced lipase or a portion thereof, or to flanking sequences in genomic DNA which are involved in regulating senescence-induced lipase gene expression. The antisense oligonucleotide may be as large as 100 nucleotides and may extend in length up to and beyond the full coding sequence for which it is antisense. The antisense oligonucleotides can be DNA or RNA or chimeric mixtures or derivatives or modified versions thereof, single stranded or double stranded.

The action of the antisense oligonucleotide may result in alteration, primarily inhibition, of senescence-induced lipase gene expression in cells. For a general discussion of antisense see: Alberts, et al., Molecular Biology of the Cell, 2nd ed., Garland

Publishing, Inc. New York, New York (1989, in particular pages 195-196, incorporated herein by reference).

The antisense oligonucleotide may be complementary to any portion of the senescence-induced lipase gene. In one embodiment, the antisense oligonucleotide may be between 6 and 100 nucleotides in length, and may be complementary to the 5'-non-coding sequence of the senescence-induced lipase sequence, for example. Antisense oligonucleotides primarily complementary to 5'-non-coding sequences are known to be effective inhibitors of expression of genes encoding transcription factors. Branch, M.A., Molec. Cell Biol., 13:4284-4290 (1993).

Preferred antisense oligonucleotides are substantially complementary to a portion of the mRNA encoding senescence-induced lipase. For example, introduction of the full length cDNA clone encoding senescence-induced lipase in an antisense orientation into a plant is expected to result in successful altered senescence-induced lipase gene expression. Moreover, introduction of partial sequences, targeted to specific portions of the senescence-induced lipase gene, can be equally effective.

The minimal amount of homology required by the present invention is that sufficient to result in sufficient complementarity to provide recognition of the specific target RNA or DNA and inhibition or reduction of its translation or function while not affecting function of other RNA or DNA molecules and the expression of other genes. While the antisense oligonucleotides of the invention comprise sequences complementary to at least a portion of an RNA transcript

of the senescence-induced lipase gene, absolute complementarity, although preferred is not required. The ability to hybridize may depend on the length of the antisense oligonucleotide and the degree of complementarity. Generally, the longer the hybridizing nucleic acid, the more base mismatches with the senescence-induced lipase target sequence it may contain and still form a stable duplex. One skilled in the art can ascertain a tolerable degree of mismatch by use of standard procedures to determine the melting temperature of the hybridized complex, for example.

The antisense RNA oligonucleotides may be generated intracellularly by transcription from exogenously introduced nucleic acid sequences. The antisense molecule may be delivered to a cell by transformation or transfection or infection with a vector, such as a plasmid or virus into which is incorporated DNA encoding the antisense senescence-

induced lipase sequence operably linked to appropriate regulatory elements, including a promoter. Within the cell the exogenous DNA sequence is expressed, producing an antisense RNA of the senescence-induced lipase gene.

Vectors can be plasmids, preferably, or may be viral or other vectors known in the art to replicate and express genes encoded thereon in plant cells or bacterial cells. The vector becomes chromosomally integrated such that it can be transcribed to produce the desired antisense senescence-induced lipase RNA. Such plasmid or viral vectors can be constructed by recombinant DNA technology methods that are standard in the art. For example, the vector may be a plasmid vector containing a replication system functional in a

prokaryotic host and an antisense oligonucleotide or polynucleotide according to the invention. Alternatively, the vector may be a plasmid containing a replication system functional in *Agrobacterium* and an antisense oligonucleotide or polynucleotide according to the invention. Plasmids that are capable of replicating in *Agrobacterium* are well known in art. See, Miki, et al., Procedures for Introducing Foreign DNA Into Plants, Methods in Plant Molecular Biology and Biotechnology,, Eds. B.R. Glick and J.E. Thompson. CRC Press (1993), PP. 67-83.

The carnation lipase gene was cloned in the antisense orientation into a plasmid vector in the following manner. The pCD plasmid, which is constructed from a pUC18 backbone and contains the 35S promoter from cauliflower mosaic virus (CaMV) followed by a multiple cloning site and an octapine synthase termination sequence was used for cloning the carnation lipase gene. The pCd-lipase (antisense) plasmid was constructed by subcloning the full length carnation lipase gene in the antisense orientation into a Hind3 site and EcoR1 site of pCd. Similarly, a pCDΔ35S-GST1-lipase (antisense) plasmid was constructed by first subcloning a PCR amplified fragment (-703 to +19 bp) of the carnation Glutathione S Transferase 1 (GST1) promoter into the BamH1 and Sal1 sites of the pCd vector. The full length carnation lipase gene was then subcloned in the antisense orientation into the Hind3 and EcoR1 sites of the construct. Another plasmid, pGdΔ35S-GST1-GUS plasmid, was contructed by first subcloning a PCR-amplified fragment (-703 to +19 bp) of the carnation Glutathione S-Transferase 1 (GST1) promoter into the BamH1 and Sal1 sites of the pCd vector. The reporter gene beta-glucuronidase (GUS) was then subcloned into the Sal1 and EcoRI sites of the

construct. The pCd-35S^2-lipase (antisense) plasmid was constructed by first subcloning a double 35S promoter (containing two copies of the CaMV 35S promoter in tandem) into the Sma1 and Hind3 sites of the pCd vector. The full length carnation lipase gene was then subcloned in the antisense orientation into the Hind3 and EcoR1 sites of the construct.

An oligonucleotide, preferably between about 6 and about 100 nucleotides in length and complementary to the target sequence of senescence-induced lipase, may be prepared by recombinant nucleotide technologies or may be synthesized from mononucleotides or shorter oligonucleotides, for example. Automated synthesizers are applicable to chemical synthesis of the oligo- and polynucleotides of the invention. Procedures for constructing recombinant nucleotide molecules in accordance with the present invention are disclosed in Sambrook, et al., In: Molecular Cloning: A Laboratory Manual, 2nd ed., Cold Spring Harbor Press, Cold Spring Harbor, N.Y. (1989), which is incorporated herein in its entirety. Oligonucleotides which encode antisense RNA complementary to senescence-induced lipase sequence can be prepared using procedures well known to those in the art. Details concerning such procedures are provided in Maniatis, T. et al., Molecular mechanisms in the Control of Gene expression, eds., Nierlich, et al., eds., Acad. Press, N.Y. (1976).

In an alternative embodiment of the invention, inhibition of expression of endogenous plant senescence-induced lipase is the result of co-suppression through over-expression of an exogenous senescence-induced lipase gene or gene fragment introduced into the plant cell. In this embodiment of the invention, a vector encoding senescence-induced lipase in the sense

orientation is introduced into the cells in the same manner as described herein for antisense molecules. Preferably, the senescence-induced lipase is operatively linked to a strong constitutive promoter, such as for example the fig wart mosaic virus promoter or CaMV35S.

Transgenic plants made in accordance with the present invention may be prepared by DNA transformation using any method of plant transformation known in the art. Plant transformation methods include direct co-cultivation of plants, tissues or cells with *Agrobacterium tumerfaciens* or direct infection (Miki, et al., Meth. in Plant Mol. Biol. and Biotechnology, (1993), p. 67-88); direct gene transfer into protoplasts or protoplast uptake (Paszkowski, et al., EMBO J., 12:2717 (1984); electroporation (Fromm, et al., Nature, 319:719 (1986); particle bombardment (Klein et al., BioTechnology, 6:559-563 (1988); injection into meristematic tissues of seedlings and plants (De LaPena, et al., Nature, 325:274-276 (1987); injection into protoplasts of cultured cells and tissues (Reich, et al., BioTechnology, 4:1001-1004 (1986)).

Generally a complete plant is obtained from the transformation process. Plants are regenerated from protoplasts, callus, tissue parts or explants, etc. Plant parts obtained from the regenerated plants in which the expression of senescence-induced lipase is altered, such as leaves, flowers, fruit, seeds and the like are included in the definition of "plant" as used herein. Progeny, variants and mutants of the regenerated plants are also included in the definition of "plant."

The present invention also provides carnation senescence-induced lipase protein encoded by the cDNA

molecule of the invention and protein which cross-reacts with antibody to the carnation protein. Such proteins have the amino acid sequence set forth in SEQ ID No:2, shown in Figure 1 or share cross reactivity with antibodies to the protein set forth in SEQ ID NO:2.

The carnation senescence-induced lipase protein or functional derivatives thereof are preferably produced by recombinant technologies, optionally in combination with chemical synthesis methods. In one embodiment of the invention the senescence-induced lipase is expressed as a fusion protein consisting of the senescence-induced lipase fused with maltose binding protein. Expression of a clone encoding the recombinant fusion protein yields a fusion protein of the expected molecular weight that hydrolyzes p-nitrophenylpalmitate, phospholipid and triacylglycerol, which is an indicator of lipase activity. The recombinant senescence-induced lipase protein shows a predominant band in Western blot analyses after immunoblotting with antibody to carnation senescence-induced lipase. The free senescence-induced lipase (50.2 Kda), which is released by treatment of the fusion protein with the protease, factor Xa, also reacts with the senescence-induced lipase antibody in Western blot analysis (Figure 3). A motif search of the senescence-induced lipase amino acid sequence shows the presence of a potential N-myristoylation site (Figure 1) for the covalent attachment of myristate via an amide linkage (See Johnson, et al., Ann. Rev. Biochem., 63: 869-914 (1994); Towler, et al., Ann. Rev. Biochem., 57:67-99 (1988); and R.J.A. Grand, Biochem. J., 258:625-638 (1989). The protein motif search also showed that the carnation senescence-induced lipase contains a sequence, ITFAGHSLGA, (SEQ ID NO:4) which is the conserved lipase consensus sequence (Table 1). The conserved lipase consensus sequence from a variety of

plants is shown in the table below.

Table 1

Plant Species	conserved Lipase Sequence
Carnation	I T F A G H S L G A (SEQ ID NO:4)
Tomato	I T F T G H S L G A (SEQ ID NO:3)
Arabidopsis	I T T C G H S L G A (SEQ ID NO:9)
Ipomoea nil	I T V T G H S L G S (SEQ ID NO:10)

The senescence-induced lipase protein of the invention was shown to possess lipase activity in both *in vitro* and *in situ* assays. For *in vitro* measurements, *p*-nitrophenylpalmitate and soybean phospholipid (40% phosphatidylcholine and 60% other phospholipids)were used as substrates, and the products of the reactions, *p*-nitrophenol and free fatty acids, respectively, were measured spectrophotometrically (Pencreac'h and Baratti, 1996; Nixon and Chan, 1979; Lin et al., 1983). Lipase activity was also measured *in vitro* by gas chromatography using a modification of the mthod described by Nixon and Chan (1979) and Lin et al. (1983). The reaction mixture contained 100 mM Tris-HCl (pH 8.0), 2.5 mM substrate (trilinolein, soybean phospholipid or dilinoleylphosphatidylcholine) and enzyme protein (100 μg) in a final volume of 100 μl. The substrates were emulsified in 5% gum arabic prior to being added to the reaction mixture. To achieve this, the substrates were dissolved in chloroform, added to the gum arabic solution and emulsified by sonication for 30 s. After emulsification, the chloroform was evaporated by a stream of N_2. The reaction was carried out at 25°C for varying periods of time up to 2 hours.

The reaction mixture was then lipid-extracted, and the free fatty acids were purfied by TLC, derivitized and quantified by GC (McKegney et al., 1995).

Lipolytic acyl hydrolase activity was measure *in situ* as described by Furukawa et al. (1983) and modified by Tsuboi et al. (1996). In this latter assay, *E. coli* transformed with the full length cDNA clone encoding senescence-induced lipase were grown in minimal salt medium supplemented with Tween 40 or Tween 60, both of which are long chain fatty acid esters, as the only source of carbon. Thus, carbon for bacterial growth was only available if the fatty acid esters were hydrolyzed by lipase. The finding that *E. coli* transformed with the scenescence-induced lipase cDNA grow in Tween 40- and Tween 60-basal medium after an initial lag phase, whereas control cultures of *E. coli* that were not transformed do not grow, confirms the lipase activity of the encoded recombinant protein (Figure 5). That is, the senescence-induced lipase releases stearate (Tween 60) and palmitate (Tween 40) to obtain the necessary carbon for growth.

"Functional derivatives" of the senescence-induced lipase protein as described herein are fragments, variants, analogs, or chemical derivatives of senescence-induced lipase, which retain at least a portion of the senescence-induced lipase activity or immunological cross reactivity with an antibody specific for senescence-induced lipase. A fragment of the senescence-induced lipase protein refers to any subset of the molecule. Variant peptides may be made by direct chemical synthesis, for example, using methods well known in the art. An analog of senescence-induced lipase refers to a non-natural protein substantially similar to either the entire protein or a fragment

thereof. Chemical derivatives of senescence-induced lipase contain additional chemical moieties not normally a part of the peptide or peptide fragment. Modifications may be introduced into the senescence-induced lipase peptide or fragment thereof by reacting targeted amino acid residues of the peptide with an organic derivatizing agent that is capable of reacting with selected side chains or terminal residues.

A senescence-induced lipase protein or peptide according to the invention may be produced by culturing a cell transformed with a nucleotide sequence of this invention (in the sense orientation), allowing the cell to synthesize the protein and then isolating the protein, either as a free protein or as a fusion protein, depending on the cloning protocol used, from either the culture medium or from cell extracts. Alternatively, the protein can be produced in a cell-free system. Ranu, et al., Meth. Enzymol., 60:459-484, (1979).

Having now generally described the invention, the same will be more readily understood through reference to the following examples which are provided by way of illustration, and are not intended to be limiting to the present invention, unless specified.

Example 1

Plant Materials Used To Isolate The Carnation Lipase cDNA

Carnation plants *(Dianthus caryophyllus* L. cv. Improved white Sim) grown and maintained in a greenhouse were used to isolate the nucleotide sequence corresponding to the senescence-induced lipase gene.

Flower tissue in the form of senescing flower petals (from different developmental stages) was collected in buffer or stored at -70°C until used.

Cytosolic lipid particles were isolated from carnation flower petals harvested just before the onset of senescence. Carnation petals (25 g/150 ml buffer) were homogenized at 4°C in homogenization buffer (50 mM Epps- 0.25 M sorbitol pH 7.4, 10 mm EDTA, 2 mM EGTA, 1 mM PMSF, 1 mM benzamadine, 10 mm amino-n-caproic acid and 4% polyvinylpolypyrrolidone) for 45 seconds in an Omnimizer and for an additional minute in a Polytron homogenizer. The homogenate was filtered through four layers of cheesecloth, and the filtrate was centifuged at 10,000 g for twenty minutes at 4°C. The supernatant was centrifuged for one hour at 250,000 g to isolate microsomal membranes. The lipid particles were obtained from the post-microsomal supernatant by collecting the particles after floatation centrifugation by the method of Hudak and Thompson, (1997), Physiol. Plant., 114:705-713. The supernatant was made 10% (w/v) with sucrose, and 23 ml of the supernatant were poured into 60 Ti Beckman centrifuge tubes, overlayed with 1.5 ml isolation buffer and centrifuged at 305,000 g for 12 hours at 4°C. The particles were removed from the isolation buffer overlayer with a Pasteur pipette. Three ml of particle suspension were loaded onto a Sepharose G-25 column equilibrated with sterile PBS (10 mM sodium phosphate buffer pH 7.5 plus 0.85% sodium chloride) and the suspension was eluted with sterile PBS. The void volume containing the particles was eluted and concentrated using a Centricon-10 filter (available from Amicon) to a protein concentration of 600 µg. The lipid particles were then used to generate antibodies in rabbits inoculated with 300 µg of the particles. The IgG titer of the blood was tested by

Western blot analysis.

Messenger RNA (mRNA) Isolation

Total RNA was isolated from petals of stage I, II, III, or IV carnation flowers essentially as described by Chomczynski and Sachi, Anal. Biochem., 162:156-159 (1987). Briefly, 15 g of petal tissue were frozen in liquid nitrogen and homogenized for 30 seconds in buffer containing 4 M guanidinium thiocyanate, 25 mM sodium citrate, pH 7.0, 0.5% sarkosyl and 0.1 M β-mercaptoethanol. 150 ml water-saturated phenol, 30 ml of chloroform and 15 ml of 2 M NaOAc, pH 4.0 were added to the homogenized sample. The sample was centrifuged at 10,000 g for ten minutes and the aqueous phase removed and nucleic acids precipitated therefrom with 150 ml isopropanol. The sample was centrifuged for ten minutes at 5,000 g and the pellet was washed once with 30 ml of 4 M LiCl, extracted with 30 ml chloroform and precipitated with 30 ml isopropanol containing 0.2 M NaOAc, pH 5.0. The RNA was dissolved in DEPC-treated water and stored at -70°C.

PolyA$^+$ mRNA was isolated from total RNA using the PolyA$^+$ tract mRNA Isolation System available from Promega. PolyA$^+$ mRNA was used as a template for cDNA synthesis using the ZAP Express® cDNA synthesis system available from Stratagene (La Jolla, Calif.)

Carnation Petal cDNA Library Screening

A cDNA library made using mRNA isolated from stage IV carnation petals was diluted to approximately 5 x 10^5 PFU/ml and immunoscreened with lipid particle antiserum. Positive cDNA clones were recovered using the ExAssist® Helper Phage/SOLR strain system and recircularized in a pBluescript® phagemid (Stratagene). A stage III

carnation petal cDNA library was also screened using a ^{32}P-labelled 19 base pair probe (5'-ACCTACTAGGTTCCGCGTC-3') (SEQ ID NO:5). Positive cDNA clones were excised from the phages and recircularized into a pBK-CMV® (Stratagene) phagemid using the method in the manufacturer's instructions. The full length cDNA (1.53 kb fragment) was inserted into the pBK-CMV vector.

Plasmid DNA Isolation, DNA Sequencing

The alkaline lysis method described by Sambrook et al., (Supra) was used to isolate plasmid DNA. The full length positive cDNA clone was sequenced using the dideoxy sequencing method. Sanger, et al., Proc. Natl. Acad. Sci. USA, 74:5463-5467. The open reading frame was compiled and analyzed using BLAST search (GenBank, Bethesda, MD) and alignment of the five most homologous proteins with the derived amino acid sequence of the encoded gene was achieved using a BCM Search Launcher: Multiple Sequence Alignments Pattern-Induced Multiple Alignment Method (See F. Corpet, Nuc. Acids Res., 16:10881-10890, (1987)). Functional motifs present in the derived amino acid sequence were identified by MultiFinder.

Expression Of The Lipase As A Fusion Protein

Phagemid pBK-CMV containing the full length senescence-induced lipase was digested with EcoRI and XbaI, which released the 1.53 Kb lipase fragment, which was subcloned into an EcoRI and XbaI digested fusion vector, pMalc (New England BioLabs). The pMalc vector containing the senescence-induced lipase, designated pMLip, was used to transform *E. coli* BL-21(DE3) cells.

The fusion protein encoded by pMLip, (fusion of the senescence-induced lipase and maltose binding protein) was isolated and purified as described in Sambrook, et

al. (Supra)and Ausubel, et al., in Current Protocols in Molecular Biology, Green Publishing Associates and Wiley Interscience, New York, (1987), 16.4.1-16.4.3. Briefly, *E. coli* BL-21 cells transformed with pMLip were resuspended in 3 ml/g lysate buffer (50 mM Tris, pH 8.0, 100 mM NaCl and 1mM EDTA) containing 8 µl of 50 mM PMSF and 80 µl of 20 mg/ml lysozyme per gram of cells and incubated for twenty minutes at room temperature with shaking. Then, 80 µl of 5% deoxycholic acid and 40 units of DNAse I were added and the cells were shaken at room temperature until the cells completely lysed. The cell debris was pelleted by centrifugation and resuspended in two volumes of lysate buffer plus 8 M urea and 0.1 mM PMSF. After one hour, seven volumes of buffer (50 mM KH_2PO_4, 1 mM EDTA and 50 mM NaCl, pH 7.0) were added to neutralize the suspension. The pH of the cell suspension was adjusted to pH 8.0 with HCl and the cell debris was pelleted. The supernatant was dialyzed against 20 mM Tris buffer, pH 8.0, 100 mM NaCl and 1 mM EDTA at 4°C overnight. The maltose binding protein-lipase fusion product (Malip) was purified using an amylose column (available from New England BioLab). Fractions containing the fusion protein were cleaved with Protease Factor Xa (1 µg/100 µg fusion protein) to separate lipase from the fusion product. Both the fusion protein and the cleaved lipase were analyzed by SDS PAGE electrophoresis and Western blots. Maltose binding protein encoded by pMalc was used as a control. The results are shown in Figure 3.

Northern Blot Hybridizations of Carnation RNA

Ten µg of total RNA isolated from flowers at stages I, II, III, IV were separated on 1% denatured formaldehyde agarose gels and immobilized on nylon membranes. The 1.53 Kb EcoRI-XbaI lipase fragment

labelled with ^{32}P-dCTP using a random primer kit (Boereinger) was used to probe the filters (7 x 10^7 cpm). The filters were washed once with 1x SSC, 0.1% SDS at room temperature and three times with 0.2x SSC, 0.1% SDS at 65°C. The filters were dried and exposed to X-ray film overnight at -70°C. The results are shown in Figure 4.

Genomic DNA Isolation And Southern Blot Hybridizations

Freshly cut carnation petals were frozen in liquid nitrogen, ground to a powder and homogenized (2 ml/g) with extraction buffer (0.1 M Tris, pH 8.2, 50 mM EDTA, 0.1M NaCl, 2% SDS, and 0.1 mg/ml proteinase K) to isolate genomic DNA. The homogenized material was incubated at 37°C for ten minutes and extracted with phenol-chloroform-isoamyl alcohol (25:24:1). DNA was precipitated with NaOAc and isopropanol. The DNA pellet was dissolved in 1 x TE, pH 8.0, re-extracted with phenol, reprecipitated and resuspended in 1 x TE, pH 8.0.

Genomic DNA was digested with restriction endonucleases (Bam HI, XbaI, XhoI, EcoRI, HindIII and SalI) separately and the digested DNA was fractionated on a 1% agarose gel. The separated DNA was blotted onto nylon membranes and hybridizations were carried out using ^{32}P-dCTP-labelled 1.53 Kb lipase fragment. Hybridization and washing were carried out under high stringency conditions (68°C))6XSSC, 2X Denhardt's reagent, 0.1% SDS)as well as low stringency conditions (42°C for hybridization and washing) (6XSSC, 5X Denhardt's reagent, 0.1% SDS). The results are shown in Figure 6. As can be seen, the lipase cDNA probe detects only one genomic fragment, indicating that the carnation lipase gene is a single copy gene.

Lipase Enzyme Assays

Lipolytic acyl hydrolase activity of the purified lipase fusion protein was assayed spectrophotometrically using *p*-nitrophenylpalmitate and soybean phospholipid as exogenous substrates. For maltose-binding protein alone, which served as a control, there was no detectable lipase activity with phospholipid as a substrate (Table 2). When *p*-nitrophenylpalmitate was used as a substrate with maltose-binding protein alone, a small amount of *p*-nitrophenol, the expected product of a lipase reaction, was detectable reflecting background levels of *p*-nitrophenol in the commercial preparation of *p*-nitrophenylpalmitate (Table 2). However, in the presence of purified lipase fusion protein, strong lipase activity manifested as the release of free fatty acids from phospholipid and *p*-nitrophenol from *p*-nitrophenylpalmitate was evident (Table 2).

Table 2

Spectrophotometric measurements of the lipolytic acyl hydrolase activity of maltose-binding protein and lipase fusion protein expressed in *E. coli* and purified by amylose column chromatography.

Two substrates, *p*-nitrophenylpalmitate and soybean phospholipid, were used.

Activities are expressed in terms of product formed (*p*-nitrophenol from *p*-nitrophenylpalmitate and free fatty acid from soybean phospholipid).

Means ± SE for n=3 replications are shown.

Protein Species	PRODUCT	
	pNPP p-nitrophenol (nmol/mg protein/min)	free fatty acid (nmol/mg protein/min)
Maltose-binding protein	0.71 ± 0.02	ND*
Lipase fusion protein	12.01 ± 1.81	46.75 ± 1.24

*ND, not detectable

In other experiments, the enzymatic activity of the lipase fusion protein was assayed by gas chromatography, a technique that enables quantitation and identification of free fatty acids released from the substrate. Trilinolein, soybean phospholipid and dilinoleylphosphatidylcholine were used as substrates, and the deesterified fatty acids were purified by thin layer chromatography prior to being analyzed by gas chromatography. In keeping with the spectrophotometric assay (Table 2), there was no detectable lipase activity for maltose-binding protein alone with either soybean phospholipid or dilinoleylphosphatidylcholine, indicating that these substrates are essentially free of deesterified fatty acids (Table 3). However, when the lipase fusion protein was used as a source of enzyme, palmitic, stearic and linoleic acids were deesterified from the soybean phospholipid extract, and linoleic acid was deesterified from dilinoleylphosphatidylcholine (Table 3). In contrast to the phospholipid substrates, detectable levels of free linoleic acid were present in trilinolein, but the levels of free linoleic acid were significantly increased in the presence of lipase fusion protein indicating that the lipase is capable of deesterifying fatty acids from triacylglycerol as well (Table 3).

Table 3

GC measurements of the lipolytic acyl hydrolase activity of maltose-binding protein and lipase fusion protein expressed in *E. coli* and purified by amylose column chromatography

Lipase Substrates fusion Protein		Products (μg/mg protein)[1]	
		Maltose-binding Protein	
Tri-linolein[2]	Linoleic acid (18:2)	15.9 ± 0.75	33.4 ± 1.58
Soybean phospholipids[3]	Palmitic acid (16:0)	ND[4]	4.80
	Stearic acid (18:0)	ND	9.68
	Linoleic acid (18:2)	ND	5.80
Dilinoleylphos-phatydilcholine[3]	Linoleic acid (18:2)	ND	20.0

[1] Reaction was allowed to proceed for 2 hours, and was not continuously linear over this period.
[2] Means ± SE for n=3 replications are shown
[3] Single experiment
[4] Not detectable

Lipase activity of the the protein obtained by expression of the lipase cDNA in *E. coli* was measured *in*

vivo as described in Tsuboi, et al., Infect. Immunol., 64:2936-2940 (1996); Wang, et al., Biotech., 9:741-746 (1995); and G. Sierra, J. Microbiol. and Serol., 23:15-22 (1957). A single colony of *E. coli* BL-21 cells transformed with pMal and another *E. coli* BL-21 colony transformed with pMLip were inoculated in basal salt medium (pH 7.0) containing (g/L): K_2HPO_4 (4.3), KH_2PO_4 (3.4), $(NH_4)SO_4$ (2.0), $MgCl_2$(0.16), $MnCl_2$.$4H_2O$(0.001), $FeSO_4$.$7H_2O$(0.0006), $CaCl_2$.$2H_2O$ (0.026), and $NaMoO_4$.$2H_2O$ (0.002). Substrate, Tween 40 (polyoxyethylenesorbitan monopalmitate) or Tween 60 (polyoxyethylenesorbitan monostearate), was added at a concentration of 1%. Growth of the bacterial cells at 37°C with shaking was monitored by measuring the absorbance at 600 nm (Figure 5). As can be seen in Figure 5, *E. coli* cells transformed with pMLip were capable of growth in the Tween40/Tween60-supplemented basal medium, after an initial lag period. However, *E. coli* cells transformed with pMal did not grow in the Tween-supplemented medium.

Example 2

Ethylene Induction of Carnation Senescence-Induced Lipase Gene

Stage II carnation flowers and carnation cuttings were treated with 0.5 ppm ethylene in a sealed chamber for 15 hours. RNA was extracted from the ethylene treated Stage II flower petals and from leaves of the treated cutting, as well as from the flower and leaves of untreated carnation flowers and cuttings as follows.

Flowers or leaves (1 flower or 5 g leaves) were ground in liquid nitrogen. The ground powder was mixed with 30 ml guanidinium buffer (4 M guanidinium

isothiocyanate, 2.5 mM NaOAc pH 8.5, 0.8% β-mercaptoethanol). The mixture was filtered through four layers of cheesecloth and centrifuged at 10,000g at 4°C for 30 minutes. The supernatant was then subjected to cesium chloride density gradient centrifugation at 26,000g for 20 hours. The pelleted RNA was rinsed with 75% ethanol, resuspended in 600 µl DEPC-treated water and the RNA precipitated at -70°C with 0.75 ml 95% ethanol and 30 µl of 3M NaOAc. Ten µg of RNA were fractionated on a 1.2% denaturing formaldehyde agarose gel and transferred to a nylon membrane. Randomly primed ^{32}P-dCTP-labelled full length carnation lipase cDNA (SEQ ID NO:1) was used to probe the membrane at 42°C overnight. The membrane was then washed once in 1X SSC containing 0.1% SDS at room temperature for 15 minutes and three times in 0.2X SSC containing 0.1% SDS at 65°C for 15 minutes each. The membrane was exposed to x-ray film overnight at -70°C.

The results are shown in Figure 9. As can be seen, transcription of carnation lipase is induced in flowers and leaves by ethylene.

Example 3

Generation of Tomato PCR Product Using Carnation Lipase Primers

A partial length senescence-induced lipase sequence from tomato genomic DNA obtained from tomato leaves was generated by nested PCR using a pair of oligonucleotide primers designed from carnation senescence-induced lipase sequence. The 5' primer is a 19-mer having the sequence, 5'- CTCTAGACTATGAGTGGGT (SEQ ID NO:7); the 3' primer is an 18-mer having the sequence,

CGACTGGCACAACCTCCA-3' (SEQ ID NO:8). Polymerase chain reaction, using genomic tomato DNA was carried out as follows.

Reaction components:

Genomic DNA	100 ng
dNTP (10 mM each)	1 μl
$MgCl_2$ (5mM)+10x buffer	5 μl
Primers 1 and 2 (20 μM each)	0.5 μl
Taq DNA polymerase	1.25 U
Reaction volume	50 μl

Reaction paramaters:

94°C for 3 min
94°C /1 min, 48°C /1 min, 72°C /2 min, for 45 cycles
72°C for 15 min .

The tomato partial length sequence obtained by PCR has the nucleotide sequence, SEQ ID NO:6 and a deduced amino acid sequence as set forth in Figure 10. The partial length sequence contains an intron (Figure 10, lower case letters) interspersed between two coding sequences. The tomato sequence contains the conserved lipase consensus sequence, ITFTGHSLGA (SEQ ID NO:3).

The tomato sequence has 53.4% sequence identity with the carnation senescence-induced lipase sequence and 43.5% sequence identity with *Arabidopsis* lipase, the latter of which has 44.3% sequence identity with the carnation sequence.

Example 4

Effect Of Chilling On Cell Membrane Integrity In Tomato Plants

Tomato plants were chilled for 48 hours at 7°C to 8°C and then returned to room temperature for 24 hours. The effect of chilling on leaves was assessed by

measuring the amount of electrolyte leakage (μMhos).

Specifically, 1g of leaf tissue was cut into a 50 ml tube, quick-rinsed with distilled water, and 40 ml of deionized water added. The tubes were capped and rotated at room temperature for 24 hours. Conductivity (μMho) readings reflecting electrolyte leakage were taken at 6 and 24 hour intervals for control and chill-injured leaf tissue. It is clear from Figure 11 that electrolyte leakage reflecting membrane damage is incurred during the rewarming period in chill injured leaf tissue.

Northern Blot Analysis Of RNA Obtained From Chilled Tomato Leaves

Total RNA was isolated from the leaves 15g of unchilled tomato plants (control) and chilled tomato plants that had been returned to room temperature for 0, 6 and 24 hours. RNA extraction was carried out as described in Example 3. 10 μg of RNA from each sample was separated on a 1.2% denaturing formaldehyde gel and transferred to a nylon membrane. The membrane was probed with ^{32}P-dCTP-labelled probe (SEQ ID NO:3)and then washed under the same conditions as described in Example 3. The results are shown in Figure 12.

As can be seen from the autoradiograph (Figure 12B) tomato lipase gene expression is induced by chilling and the pattern of gene induction correlates with increased electrolyte leakage in chill injured leaves (Figure 11).

What is claimed is:

Claim 1. An isolated DNA molecule encoding senescence-induced lipase, wherein the DNA molecule hybridizes under low stringency conditions with SEQ ID NO:1, or a functional derivative of the isolated DNA molecule which hybridizes with SEQ ID NO:1.

Claim 2. The isolated DNA molecule of claim 1 wherein the DNA molecule has the nucleotide sequence of SEQ ID NO:1.

Claim 3. The isolated DNA molecule of claim 1 wherein the isolated DNA molecule contains the nucleotide sequence of SEQ ID NO:4.

Claim 4. An isolated senescence-induced lipase encoded by a nucleotide sequence which hybridizes under low stringency conditions with SEQ ID NO:1, or a functional derivative of the senescence-induced lipase.

Claim 5. The senescence-induced lipase of claim 4 wherein the lipase has the amino acid sequence SEQ ID NO:2.

Claim 6. A vector for transformation of plant cells comprising

(a) an antisense oligo- or polynucleotide substantially complementary to (1) at least a portion of one strand of a DNA molecule encoding senescence-induced lipase, wherein the DNA molecule encoding senescence-

induced lipase hybridizes under low stringency conditions with SEQ ID NO:1, or (2) at least a portion of an RNA sequence encoded by the DNA molecule encoding senescence-induced lipase; and

(b) regulatory sequences operatively linked to the antisense oligo- or polynucleotide such that the antisense oligo- or polynucleotide is expressed in a plant cell into which it is transformed.

Claim 7. The vector according to claim 6 wherein the regulatory sequences comprise a promoter and a transcription termination region.

Claim 8. The vector according to claim 6 wherein the regulatory sequences comprise a constitutive promoter.

Claim 9. The vector according to claim 6 wherein the regulatory sequences comprise a plant tissue-specific promoter.

Claim 10. The vector according to claim 6 wherein the regulatory sequences comprise a senescence-induced plant promoter.

Claim 11. The vector according to claim 6 wherein the regulatory sequences comprise a viral promoter.

Claim 12. The vector according to claim 11 wherein the regulatory sequences comprise a constitutive

promoter.

Claim 13. An antisense oligonucleotide or polynucleotide encoding an RNA molecule which is substantially complementary to at least a portion of an RNA transcript of a plant senescence-induced lipase gene, wherein said plant gene hybridizes under low stringency conditions with SEQ ID NO:1.

Claim 14. The antisense oligonucleotide or polynucleotide according to claim 13 wherein the oligonucleotide or polynucleotide comprises about six to about 100 nucleotides.

Claim 15. The antisense oligonucleotide or polynucleotide according to claim 13 wherein the coding region of the plant gene has the nucleotide sequence SEQ ID NO:1.

Claim 16. The antisense oligonucleotide or polynucleotide according to claim 13 wherein the plant gene is a carnation gene.

Claim 17. The antisense oligonucleotide or polynucleotide according to claim 13 wherein the plant gene is a tomato gene.

Claim 18. The antisense oligonucleotide or polynucleotide according to claim 13 wherein the plant gene is a green bean gene.

Claim 19. The antisense oligonucleotide or polynucleotide according to claim 13 wherein the antisense oligonucleotide or polynucleotide is substantially complementary to at least a portion of the 5'-non-coding region of the RNA transcript.

Claim 20. A vector comprising

(a) a DNA molecule encoding senescence-induced lipase, wherein the DNA molecule hybridizes under low stringency conditions with SEQ ID NO:1; and

(b) regulatory sequences operatively linked to the DNA molecule such that the DNA molecule is expressed in a plant cell into which it is transformed.

Claim 21. A bacterial cell transformed with the vector according to claim 20.

Claim 21. A plant cell transformed with the vector according to claim 6.

Claim 22. A plant and progeny thereof generated from a plant cell transformed with the vector according to claim 6.

Claim 23. A plant, plant part or plant progeny according to claim 22.

Claim 24. A method for inhibiting the expression of endogenous senescence-induced lipase in a plant, said

method comprising

(1) integrating into the genome of the plant a vector comprising

(A) an antisense oligo- or polynucleotide substantially complementary to (i) at least a portion of one strand of a DNA molecule encoding the endogenous senescence-induced lipase, wherein the DNA molecule encoding the endogenous senescence-induced lipase hybridizes with SEQ ID NO:1, or (ii) at least a portion of an RNA sequence encoded by the endogenous senescence-induced lipase gene; and

(B) regulatory sequences operatively linked to the antisense oligo- or polynucleotide such that the antisense oligo- or polynucleotide is expressed; and

(2) growing said plant, whereby said antisense oligo- or polynucleotide is transcribed and binds to said RNA sequence, whereby expression of said senescence-induced lipase gene is inhibited.

Claim 25. The method according to claim 24 wherein the portion of the DNA or the portion of the RNA to which the antisense oligo- or polynucleotide is substantially complementary comprises 5'-non-coding sequences.

Claim 26. The method according to claim 24 wherein said inhibition results in altered senescence of the plant.

Claim 27. The method according to claim 24 wherein said inhibition results in increased resistance of said plant to environmental stress-induced senescence.

Claim 28. The method according to claim 24 wherein the regulatory sequences comprise a constitutive promoter active in the plant.

Claim 29. The method according to claim 24 wherein the regulatory sequences comprise a constitutive promoter.

Claim 30. The method according to claim 24 wherein the regulatory sequences comprise a tissue specific promoter active in the plant.

Claim 31. The method according to claim 24 wherein the regulatory sequences comprise a senescence-induced promoter active in the plant.

Claim 32. The method according to claim 24 wherein said plant is selected from the group consisting of fruit bearing plants, flowering plants and vegetables.

Claim 33. The method according to claim 24 wherein the plant is a tomato.

Claim 34. The method according to claim 24 wherein the plant is a carnation.

Claim 35. A method for inhibiting the expression of an endogenous senescence-induced lipase gene in a plant cell, said method comprising

(1) integrating into the genome of at least one

cell of the plant a vector comprising

(A) an isolated DNA molecule encoding exogenous senescence-induced lipase, wherein the DNA molecule hybridizes under low stringency conditions with SEQ ID NO:1., or a functional derivative of the isolated DNA molecule which hybridizes with SEQ ID; and

(B) regulatory sequences operatively linked to the DNA molecule such that the exogenous senescence-induced lipase encoded thereby is expressed; and

(2) growing said plant, whereby said DNA molecule is over-expressed and the endogenous senescence-induced lipase gene is inhibited by exogenous senescence-induced lipase.

Claim 36. The method according to claim 35 wherein the regulatory sequences comprise a constitutive promoter.

Claim 37. A method of altering age-related senescence and environmental stress-related senescence in a plant, said method comprising

(1) integrating into the genome of the plant a vector comprising

(A) an antisense oligo- or polynucleotide substantially complementary to (i) at least a portion of one strand of a DNA molecule encoding the endogenous senescence-induced lipase, wherein the DNA molecule encoding the endogenous senescence-induced lipase hybridizes with SEQ ID NO:1, or (ii) at least a portion of an RNA sequence encoded by the endogenous senescence-induced lipase gene; and

(B) regulatory sequences operatively linked to the antisense oligo- or polynucleotide such that the

antisense oligo- or polynucleotide is expressed; and

(2) growing said plant, whereby said antisense oligo- or polynucleotide is transcribed and binds to said RNA sequence, whereby expression of said senescence-induced lipase gene is inhibited.

Claim 38. A transgenic plant cell comprising a vector according to claim 6.

Claim 39. A transgenic plant cell comprising a vector according to claim 20.

Claim 40. A plasmid comprising a replication system functional in a prokaryotic host and an antisense oligonucleotide or polynucleotide according to claim 13.

Claim 41. A plasmid comprising a replication system functional in *Agrobacterium* and an antisense oligonucleotide or polynucleotide according to claim 13.

Claim 42. A plant and progeny thereof derived from a cell having inhibited or reduced expression of senescence-induced lipase, said cell comprising a vector according to claim 6.

Claim 43. A plant and progeny thereof derived from a cell having inhibited or reduced expression of senescence-induced lipase, wherein said cell is produced by

(1) integrating into the genome of the cell a

vector comprising

(A) an antisense oligo- or polynucleotide substantially complementary to (i) at least a portion of one strand of a DNA molecule encoding the endogenous senescence-induced lipase, wherein the DNA molecule encoding the endogenous senescence-induced lipase hybridizes with SEQ ID NO:1, or (ii) at least a portion of an RNA sequence encoded by the endogenous senescence-induced lipase gene; and

(B) regulatory sequences operatively linked to the antisense oligo- or polynucleotide such that the antisense oligo- or polynucleotide is expressed; and

(2) growing said cell, whereby said antisense oligo- or polynucleotide is transcribed and binds to said RNA sequence, whereby expression of said senescence-induced lipase gene is inhibited.

Claim 44. The plant and progeny according to claim 43 wherein the plant is a tomato.

Claim 45. The plant and progent according to claim 43 wherein the plant is a carnation.

Claim 46. A method of inhibiting seed aging, said method comprising

(1) integrating into the genome of a plant a vector comprising

(A) an antisense oligo- or polynucleotide substantially complementary to (i) at least a portion of one strand of a DNA molecule encoding an endogenous aging-induced lipase, wherein DNA encoding said endogenous aging-induced lipase hybridizes with SEQ ID NO.:1, or (ii) at least a portion of an RNA sequence transcribed from a DNA molecule encoding an endogenous aging-induced lipase; and

(B) regulatory sequences operatively linked

to the antisense oligo- or polynucleotide; and

(2) growing said plant, whereby said antisense oligo- or polynucleotide is transcribed and binds to said RNA sequence and expression of said aging-induced lipase gene is inhibited.

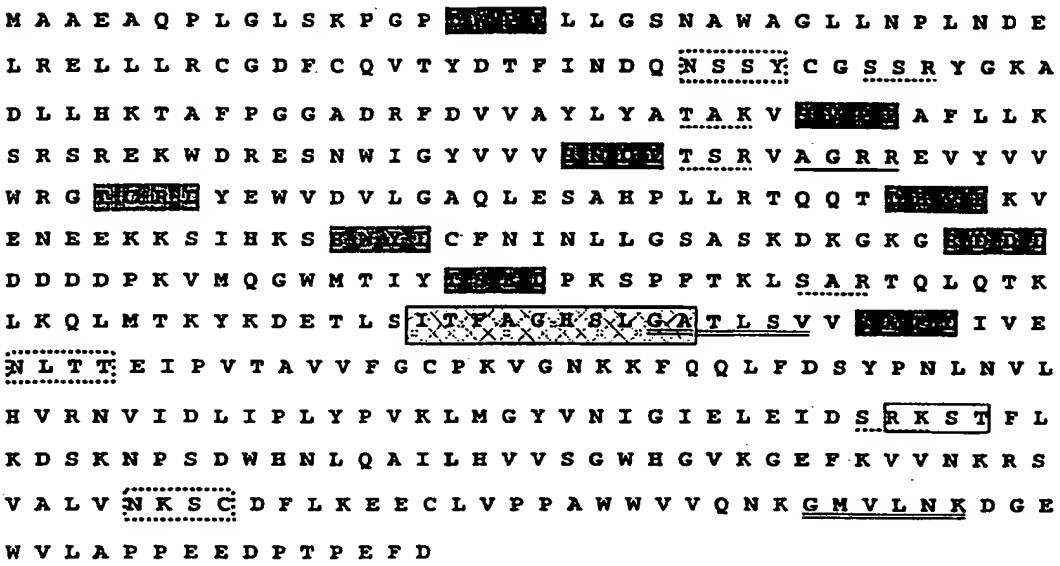

M A A E A Q P L G L S K P G P L L G S N A W A G L L N P L N D E
L R E L L L R C G D F C Q V T Y D T F I N D Q N S S Y C G S S R Y G K A
D L L H K T A F P G G A D R F D V V A Y L Y A T A K V A F L L K
S R S R E K W D R E S N W I G Y V V V T S R V A G R R E V Y V V
W R G Y E W V D V L G A Q L E S A H P L L R T Q Q T K V
E N E E K K S I H K S C F N I N L L G S A S K D K G K G
D D D D P K V M Q G W M T I Y P K S P F T K L S A R T Q L Q T K
L K Q L M T K Y K D E T L S I T F A G H S L G A T L S V V I V E
N L T T E I P V T A V V F G C P K V G N K K F Q Q L F D S Y P N L N V L
H V R N V I D L I P L Y P V K L M G Y V N I G I E L E I D S R K S T F L
K D S K N P S D W H N L Q A I L H V V S G W H G V K G E F K V V N K R S
V A L V N K S C D F L K E E C L V P P A W W V V Q N K G M V L N K D G E
W V L A P P E E D P T P E F D

Figure 1

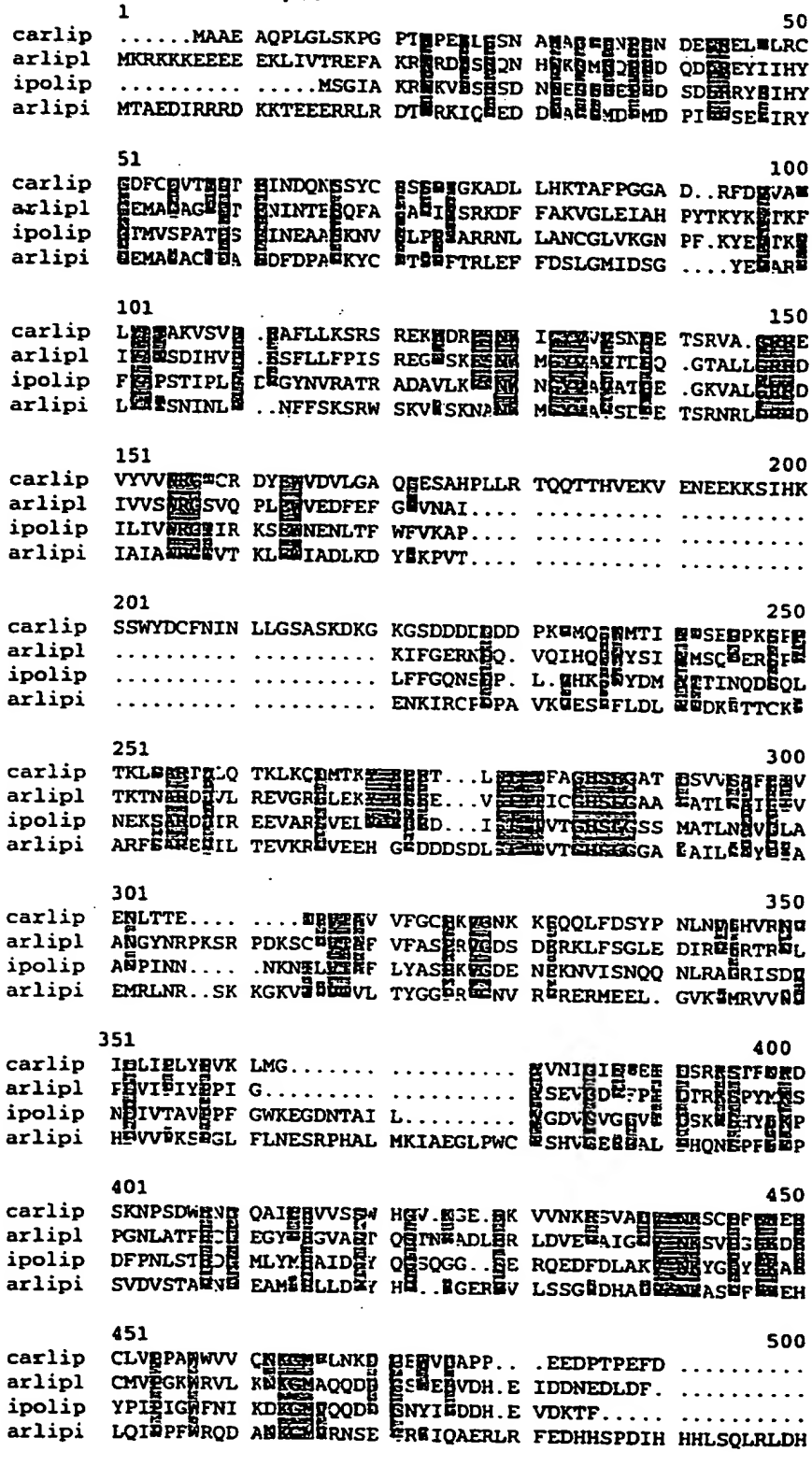


Figure 2

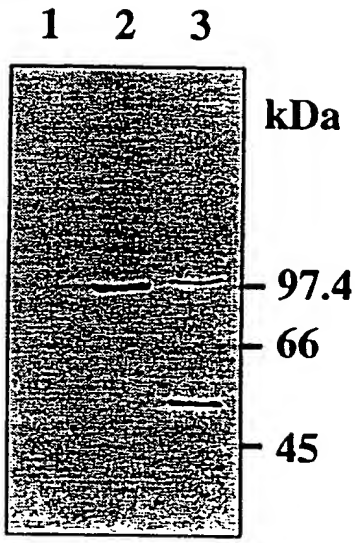
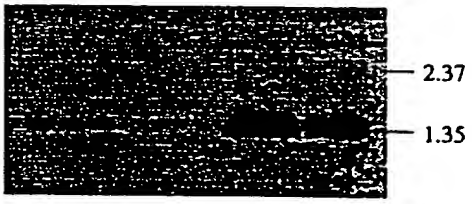


Figure 3

Figure 4A

Figure 4B



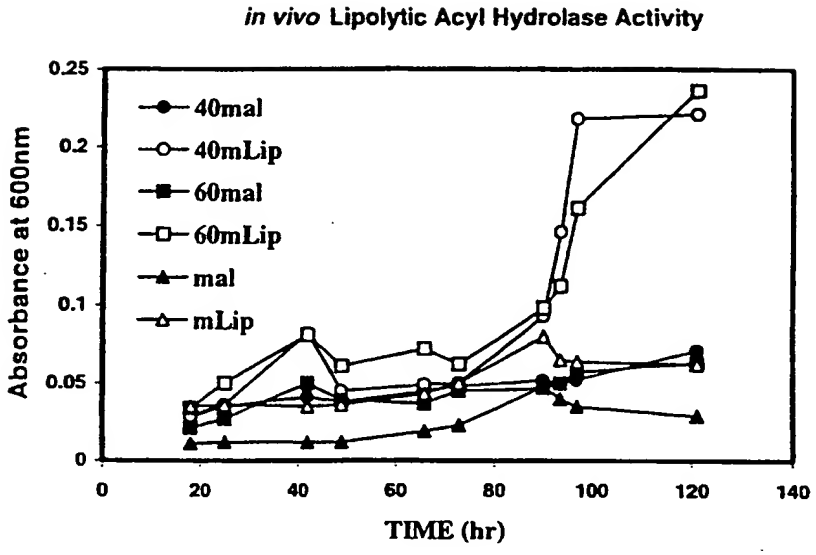

in vivo Lipolytic Acyl Hydrolase Activity
Absorbance at 600nm
0.25
0.2
0.15
0.1
0.05
0
40mal
40mLip
60mal
60mLip
mal
mLip
0
20
40
60
80
100
120
140
TIME (hr)

Figure 5

Figure 6A

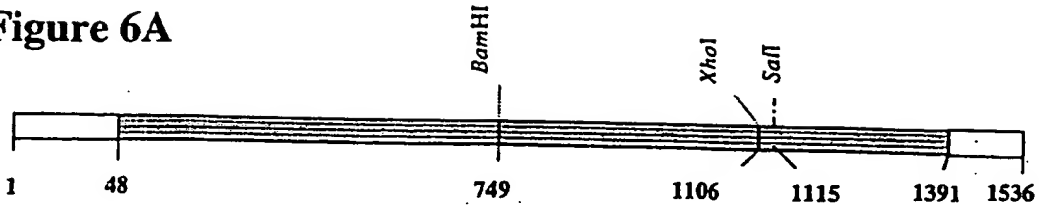
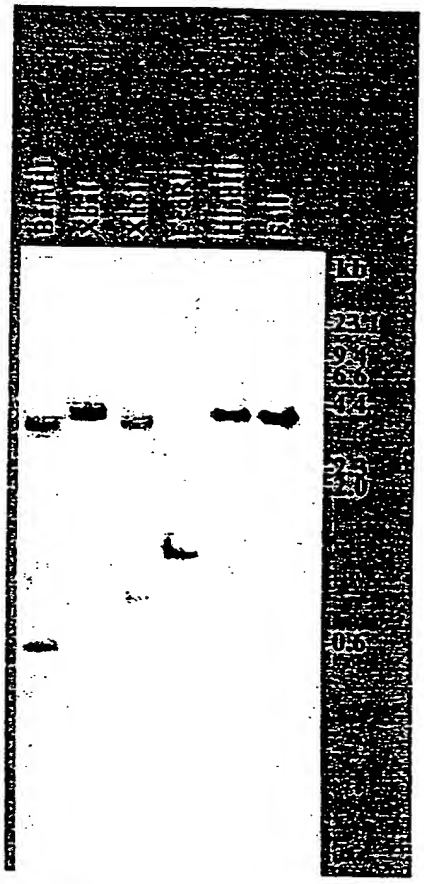


Figure 6B



GCACGAGCCATTCCAAAACTCCTTACACCACTCAAAACTATTCCAACATGGCTGCAGAAGCCCAACCTTTAGGCCTCTC
AAAGCCCGGCCCAACATGGCCCGAACTCCTCGGGTCCAACGCTTGGGCCGGGCTACTAAACCCGCTCAACGATGAGCTC
CGTGAGCTCCTCCTACGCTGCGGGGACTTCTGCCAGGTGACATACGACACCTTCATAAACGACCAGAACTCGTCCTACT
GCGGCAGCAGCCGCTACGGGAAGGCGGACCTACTTCATAAGACCGCCTTCCCGGGGGGCGCAGACCGGTTTGACGTGGT
GGCGTACTTGTACGCCACTGCGAAGGTCAGCGTCCCAgAGGCGTTTCTGCTGAAGTCGAGGTCGAGGGAGAAGTGGGAT
AGGGAATCGAATTGGATTGGGTATGTCGTGGTGTCGAATGACGAGACGAGTCGGGTGGCGGGACGAAgGGAGGTGTATG
TGGTGTGGAGAGGGACTTGTAgGGATTATGAGTGGGTTGATGTTCTTGGTGCTCAACTTGAGTCTGCTCATCCTTTGTT
ACGCACTCAACAAACTACTCATGTTGAAAAGGTGGAAAATGAGGAAAAGAAGAGCATTCATAAATCAAGTTGGTACGAC
TGTTTCAATATCAACCTACTAgGTTCCGCGTCCAAAGACAAAGGAAAAGGAAgCGACGACGACGATGATGACGACCCCA
AAGTGATGCAAGGTTGGATGACAATATACACATCGGAGGATCCCAAATCACCCTTCACAAAACTAAGTGCAAGAACACA
ACTTCAGACCAAACTCAAACAACTAATGACAAAATACAAAGACGAAACCCTAAGCATAACATTCGCCGGTCACAGCCTA
GGCGCGACACTATCAGTCGTGAGCGCCTTCGACATAGTGGAGAATCTCACGACCGAGATCCCAGTCACGGCCGTGGTCT
TCGGGTGCCCAAAAGTAGGCAACAAAAAATTCCAACAACTCTTCGACTCGTACCCAAACCTAAATGTCCTCCATGTAAG
GAATGTCATCGACCTGATCCCTCTGTATCCCGTGAAACTCATGGGTTACGTGAACATAGGAATCGAGCTGGAGATCGAC
TCGAGGAAGTCGACCTTTCTAAAGGACTCGAAAAACCCGAGTGATTGGCATAATTTGCAAGCAATATTGCATGTTGTAA
GTGGTTGGCATGGGGTTAAGGGGGAGTTTAAGGTTGTAAATAAGAGAAGTGTTGCATTGGTTAATAAGTCATGTGATTT
TCTTAAGGAAGAATGTTTGGTTCCTCCAGCTTGGTGGGTTGTGCAGAACAAAGGGATGGTTTTGAATAAGGATGGTGAG
TGGGTTTTGGCTCCTCCTGAGGAAGATCCTACTCCTGAATTTGATTGATAATATTTCATCATGTTTTATATTTTTATAA
ATTTTACTAAATTTACATGACAATTTATGGGACTAAGTTACTTATTTATATGTTTATTATATTTGAAATGTGTTTTAAG
TTACATAAAATTGCAATTAGTTTTAAAAAAAAAAAA

———— uncoding region of cDNA clone

Figure 7

```
Met Ala Ala Glu Ala Gln Pro Leu Gly Leu Ser Lys Pro Gly Pro Thr Trp Pro Glu Leu
 1               5                  10                  15                  20

Leu Gly Ser Asn Ala Trp Ala Gly Leu Leu Asn Pro Leu Asn Asp Glu Leu Arg Glu Leu
            21                  25                  30                  35

Leu Leu Arg Cys Gly Asp Phe Cys Gln Val Thr Tyr Asp Thr Phe Ile Asn Asp Gln Asn
            41                  45                  50                  55

Ser Ser Tyr Cys Gly Ser Ser Arg Tyr Gly Lys Ala Asp Leu Leu His Lys Thr Ala Phe
            61                  65                  70                  75

Pro Gly Gly Ala Asp Arg Phe Asp Val Val Ala Tyr Leu Tyr Ala Thr Ala Lys Val Ser
            81                  85                  90                  95

Val Pro Glu Ala Phe Leu Leu Lys Ser Arg Ser Arg Glu Lys Trp Asp Arg Glu Ser Asn
           101                 105                 110                 115

Trp Ile Gly Tyr Val Val Val Ser Asn Asp Glu Thr Ser Arg Val Ala Gly Arg Arg Glu
           121                 125                 130                 135

Val Tyr Val Val Trp Arg Gly Thr Cys Arg Asp Tyr Glu Trp Val Asp Val Leu Gly Ala
           141                 145                 150                 155

Gln Leu Glu Ser Ala His Pro Leu Leu Arg Thr Gln Gln Thr Thr His Val Glu Lys Val
           161                 165                 170                 175

Glu Asn Glu Glu Lys Lys Ser Ile His Lys Ser Ser Trp Tyr Asp Cys Phe Asn Ile Asn
           181                 185                 190                 195

Leu Leu Gly Ser Ala Ser Lys Asp Lys Gly Lys Gly Ser Asp Asp Asp Asp Asp Asp Asp
           201                 205                 210                 215

Pro Lys Val Met Gln Gly Trp Met Thr Ile Tyr Thr Ser Glu Asp Pro Lys Ser Pro Phe
           221                 225                 230                 235

Thr Lys Leu Ser Ala Arg Thr Gln Leu Gln Thr Lys Leu Lys Gln Leu Met Thr Lys Tyr
           241                 245                 250                 255

Lys Asp Glu Thr Leu Ser Ile Thr Phe Ala Gly His Ser Leu Gly Ala Thr Leu Ser Val
           261                 265                 270                 275

Val Ser Ala Phe Asp Ile Val Glu Asn Leu Thr Thr Glu Ile Pro Val Thr Ala Val Val
           281                 285                 290                 295

Phe Gly Cys Pro Lys Val Gly Asn Lys Lys Phe Gln Gln Leu Phe Asp Ser Tyr Pro Asn
           301                 305                 310                 315

Leu Asn Val Leu His Val Arg Asn Val Ile Asp Leu Ile Pro Leu Tyr Pro Val Lys Leu
           321                 325                 330                 335

Met Gly Tyr Val Asn Ile Gly Ile Glu Leu Glu Ile Asp Ser Arg Lys Ser Thr Phe Leu
           341                 345                 350                 355

Lys Asp Ser Lys Asn Pro Ser Asp Trp His Asn Leu Gln Ala Ile Leu His Val Val Ser
           361                 365                 370                 375

Gly Trp His Gly Val Lys Gly Glu Phe Lys Val Val Asn Lys Arg Ser Val Ala Leu Val
           381                 385                 390                 395

Asn Lys Ser Cys Asp Phe Leu Lys Glu Glu Cys Leu Val Pro Pro Ala Trp Trp Val Val
           401                 405                 410                 415

Gln Asn Lys Gly Met Val Leu Asn Lys Asp Gly Glu Trp Val Leu Ala Pro Pro Glu Glu
           421                 425                 430                 435

Asp Pro Thr Pro Glu Phe Asp
           441                 445
```

Figure 8

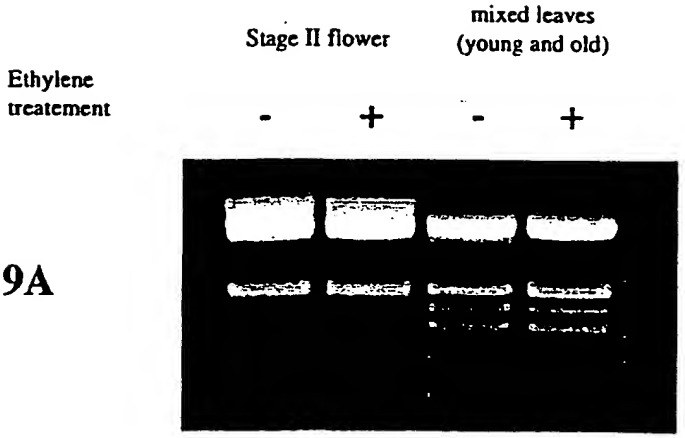


Figure 9A

Figure 9B

A Tomato Lipase PCR Product

A Genomic DNA PCR product---TLip

(PCR primer-1)
CTCTAGACTATGAGTGGGTGGATGTTTTAGGTGCTCGTCCTGATTCAGCTGACTCTCTTCTTCATCCTAAATCTCTCCAA
D Y E W V D V L G A R P D S A D S L L H P K S L Q

AAAGGCATTAACAACAAGAACGATGAGGATGAGGACGAGGACGAGGATGAGATCAAAGTAATGGATGGGTGGCTTAAGAT
K G I N N K N D E D E D E D E D E D E I K V M D G W L K I

CTACGTCTCAAGTAACCCGAAGTCGTCTTTCACGAGACTAAGTGCAAGAGAACAACTTCAAGCAAAGATTGAAAAGTTAA
Y V S S N P K S S F T R L S A R E Q L Q A K I E K L R

GAAATGAGTATAAAGATGAGAATTTGAGCATAACTTTTACAGGGCATAGTCTTGGTGCTAGCTTAGCTGTTTTAGCTTCA
N E Y K D E N L S [redacted] S L A V L A S

TTTGATGTGGTTGAAAATGGTGTGCCAGTTGATATTCCAGTATCTGCAATTGTATTTGGTAGTCCACAAGTTGGGAATAA
F D V V E N G V P V D I P V S A I V F G S P Q V G N K

GGCATTCAATGAAAGAATCAAGAAATTCTCAAACTTGAATATCTTACATGTTAAGAACAAGATTGATCTCATTACCCTTT
A F N E R I K K F S N L N I L H V K N K I D L I T L Y

ACCCAAGTGCTCTGTTTGGGTATGTGAATTCAGgtattgaaggaaaagatcattacaattttgagctagatttctcatat
P S A L F G Y V N S G

cgtcacactcaactaacagttattatatgagaaagtcactttctttgtgaaaaaattgaatcaacttttggaaataatag

tagttgagtgaccatatgagaaatcaacactctactaactttatgctataagagaataggttaaggtccatatgtttata

ctgtctgttcaattagaatcataaaagtattactagttaaatttgactacaatcttatgtagacatgaataaaataaatc

ctacataaataagatttcctacaactttaatgattcttcaacagGTATAGAGCTAGTCATCGATAGCAGAAAGTCTCCGA
I E L V I D S R K S P S

(PCR primer-3)
GTTTAAAGGATTCAAAAGACATGGGCGACTGGCACAACCTCCA
L K D S K D M G D W H N L

Figure 10

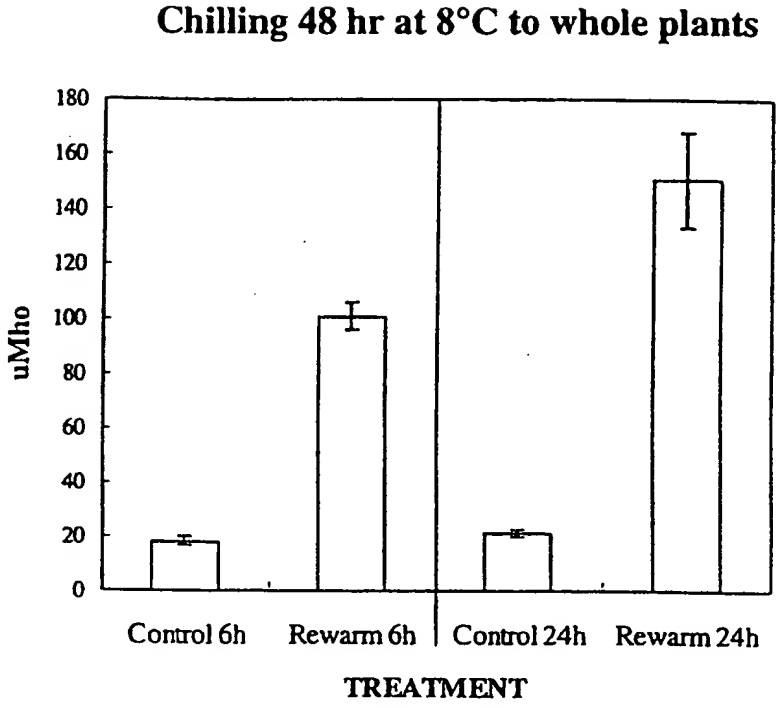

Chilling 48 hr at 8°C to whole plants
180
160
140
120
100
80
60
40
20
0
uMho
Control 6h
Rewarm 6h
Control 24h
Rewarm 24h
TREATMENT

Figure 11

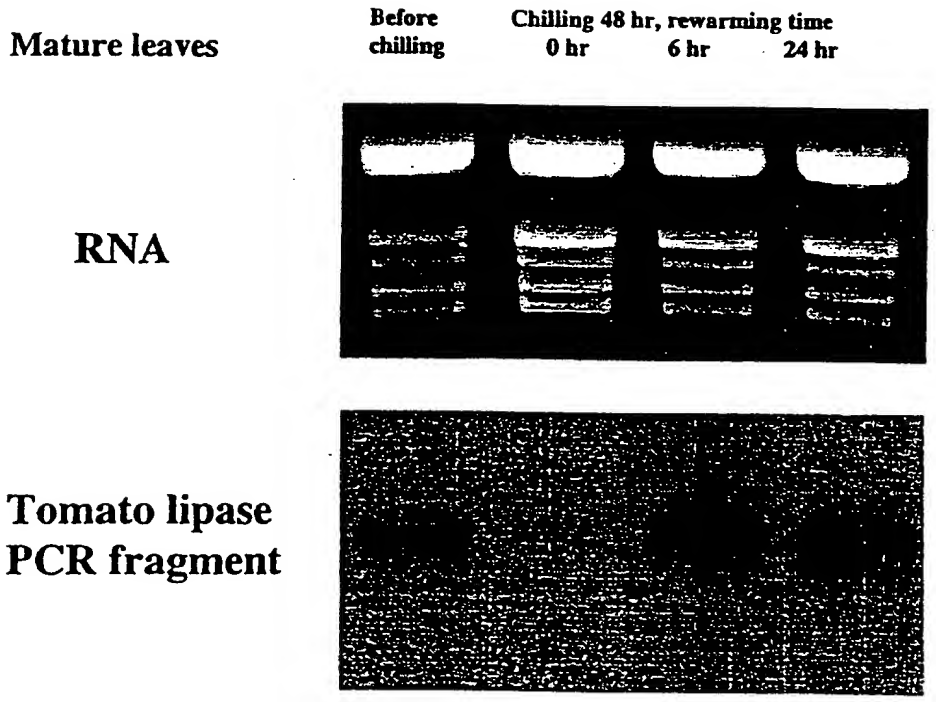
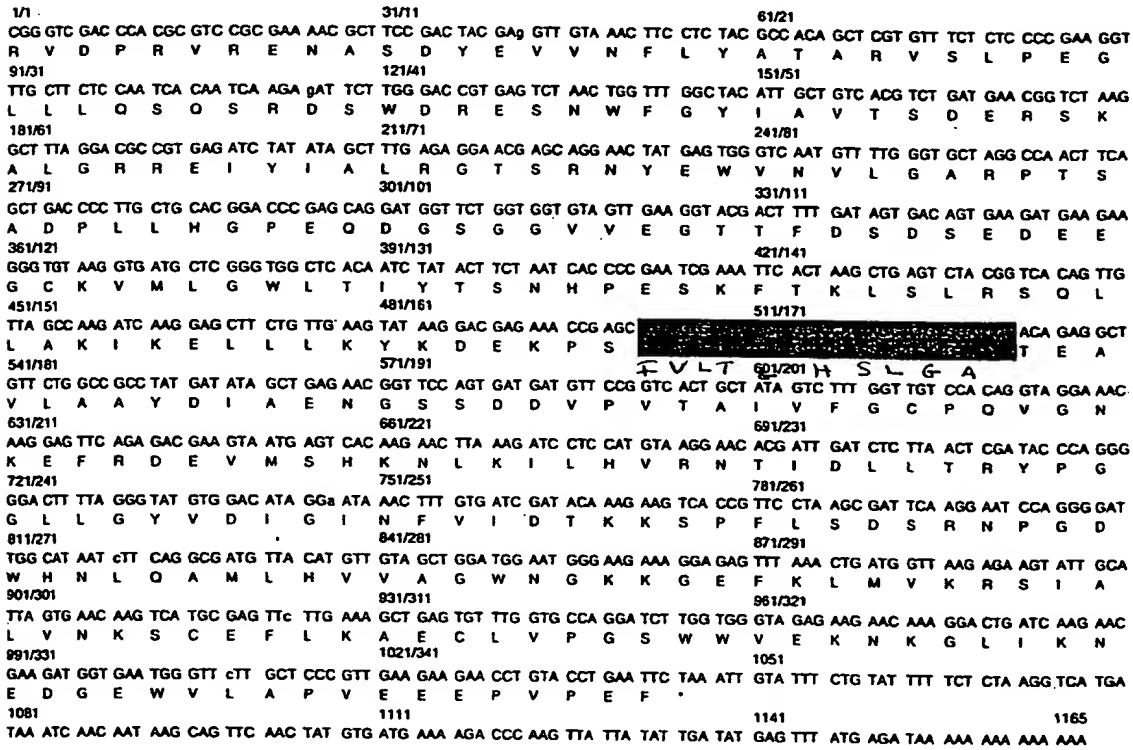

Mature leaves
Before chilling
Chilling 48 hr, rewarming time
0 hr
6 hr
24 hr
RNA
Tomato lipase PCR fragment

Figure 12

An Arabidopsis EST (GenBank Acc. # : N38227) that is 55.5 % identical with a 364 aa overlap region of the Carnation Lipase



Note: The identity of nucleotides indicated in lower case needs to be confirmed.

Figure 13

SEQUENCE LISTING

<110> Senesco, Inc.

<120> DNA ENCODING A PLANT LIPASE, TRANSGENIC PLANTS AND A METHOD FOR CONTROLLING SENESCENCE IN PLANTS

<130> 10799/7

<140>
<141>

<150> 09/250,280
<151> 1999-02-16

<150> 09/105,812
<151> 1998-06-26

<160> 17

<170> PatentIn Ver. 2.1

<210> 1
<211> 1537
<212> DNA
<213> Dianthus caryophyllus

<400> 1
gcacgagcca ttccaaaact ccttacacca ctcaaaacta ttccaacatg gctgcagaag 60
cccaaccttt aggcctctca aagcccggcc caacatggcc cgaactcctc gggtccaacg 120
cttgggccgg gctactaaac ccgctcaacg atgagctccg tgagctcctc ctacgctgcg 180
gggacttctg ccaggtgaca tacgacacct tcataaacga ccagaactcg tcctactgcg 240
gcagcagccg ctacgggaag gcggacctac ttcataagac cgccttcccg gggggcgcag 300
accggtttga cgtggtggcg tacttgtacg ccactgcgaa ggtcagcgtc ccagaggcgt 360
ttctgctgaa gtcgaggtcg agggagaagt gggataggga atcgaattgg attgggtatg 420
tcgtggtgtc gaatgacgag acgagtcggg tggcgggacg aagggaggtg tatgtggtgt 480
ggagagggac ttgtagggat tatgagtggg ttgatgttct tggtgctcaa cttgagtctg 540
ctcatccttt gttacgcact caacaaacta ctcatgttga aaaggtggaa aatgaggaaa 600
agaagagcat tcataaatca agttggtacg actgtttcaa tatcaaccta ctaggttccg 660
cgtccaaaga caaaggaaaa ggaagcgacg acgacgatga tgacgacccc aaagtgatgc 720
aaggttggat gacaatatac acatcggagg atcccaaatc acccttcaca aaactaagtg 780
caagaacaca acttcagacc aaactcaaac aactaatgac aaaatacaaa gacgaaaccc 840
taagcataac attcgccggt cacagcctag gcgcgacact atcagtcgtg agcgccttcg 900
acatagtgga gaatctcacg accgagatcc cagtcacggc cgtggtcttc gggtgcccaa 960
aagtaggcaa caaaaaattc caacaactct tcgactcgta cccaaaccta aatgtcctcc 1020
atgtaaggaa tgtcatcgac ctgatccctc tgtatcccgt gaaactcatg ggttacgtga 1080
acataggaat cgagctggag atcgactcga ggaagtcgac ctttctaaag gactcgaaaa 1140
acccgagtga ttggcataat ttgcaagcaa tattgcatgt tgtaagtggt tggcatgggg 1200
ttaaggggga gtttaaggtt gtaaataaga gaagtgttgc attggttaat aagtcatgtg 1260
attttcttaa ggaagaatgt ttggttcctc cagcttggtg ggttgtgcag aacaaaggga 1320
tggttttgaa taaggatggt gagtgggttt tggctcctcc tgaggaagat cctactcctg 1380
aatttgattg ataatatttc atcatgtttt atatttttat aaattttact aaatttacat 1440
gacaatttat gggactaagt tacttattta tatgtttatt atatttgaaa tgtgttttaa 1500
gttacataaa attgcaatta gttttaaaaa aaaaaaa 1537

<210> 2
<211> 447

<212> PRT
<213> Dianthus caryophyllus

<400> 2

```
Met Ala Ala Glu Ala Gln Pro Leu Gly Leu Ser Lys Pro Gly Pro Thr
  1               5                  10                  15

Trp Pro Glu Leu Leu Gly Ser Asn Ala Trp Ala Gly Leu Leu Asn Pro
             20                  25                  30

Leu Asn Asp Glu Leu Arg Glu Leu Leu Leu Arg Cys Gly Asp Phe Cys
         35                  40                  45

Gln Val Thr Tyr Asp Thr Phe Ile Asn Asp Gln Asn Ser Ser Tyr Cys
     50                  55                  60

Gly Ser Ser Arg Tyr Glu Lys Ala Asp Leu Leu His Lys Thr Ala Phe
 65                  70                  75                  80

Pro Gly Gly Ala Asp Arg Phe Asp Val Val Ala Tyr Leu Tyr Ala Thr
                 85                  90                  95

Ala Lys Val Ser Val Pro Glu Ala Phe Leu Leu Lys Ser Arg Ser Arg
            100                 105                 110

Glu Lys Trp Asp Arg Glu Ser Asn Trp Ile Gly Tyr Val Val Val Ser
        115                 120                 125

Asn Asp Glu Thr Ser Arg Val Ala Gly Arg Arg Glu Val Tyr Val Val
    130                 135                 140

Trp Arg Gly Thr Cys Arg Asp Tyr Glu Trp Val Asp Val Leu Gly Ala
145                 150                 155                 160

Gln Leu Glu Ser Ala His Pro Leu Leu Arg Thr Gln Gln Thr Thr His
                165                 170                 175

Val Glu Lys Val Glu Asn Glu Glu Lys Lys Ser Ile His Lys Ser Ser
            180                 185                 190

Trp Tyr Asp Cys Phe Asn Ile Asn Leu Leu Gly Ser Ala Ser Lys Asp
        195                 200                 205

Lys Gly Lys Gly Ser Asp Asp Asp Asp Asp Asp Asp Pro Lys Val Met
    210                 215                 220

Gln Gly Trp Met Thr Ile Tyr Thr Ser Glu Asp Pro Lys Ser Pro Phe
225                 230                 235                 240

Thr Lys Leu Ser Ala Arg Thr Gln Leu Gln Thr Lys Leu Lys Gln Leu
                245                 250                 255

Met Thr Lys Tyr Lys Asp Glu Thr Leu Ser Ile Thr Phe Ala Gly His
            260                 265                 270

Ser Leu Gly Ala Thr Leu Ser Val Val Ser Ala Phe Asp Ile Val Glu
        275                 280                 285
```

```
Asn Leu Thr Thr Glu Ile Pro Val Thr Ala Val Val Phe Gly Cys Pro
    290                 295                 300

Lys Val Gly Asn Lys Lys Phe Gln Gln Leu Phe Asp Ser Tyr Pro Asn
305                 310                 315                 320

Leu Asn Val Leu His Val Arg Asn Val Ile Asp Leu Ile Pro Leu Tyr
                325                 330                 335

Pro Val Lys Leu Met Gly Tyr Val Asn Ile Gly Ile Glu Leu Glu Ile
            340                 345                 350

Asp Ser Arg Lys Ser Thr Phe Leu Lys Asp Ser Lys Asn Pro Ser Asp
        355                 360                 365

Trp His Asn Leu Gln Ala Ile Leu His Val Val Ser Gly Trp His Gly
    370                 375                 380

Val Lys Gly Glu Phe Lys Val Val Asn Lys Arg Ser Val Ala Leu Val
385                 390                 395                 400

Asn Lys Ser Cys Asp Phe Leu Lys Glu Glu Cys Leu Val Pro Pro Ala
                405                 410                 415

Trp Trp Val Val Gln Asn Lys Gly Met Val Leu Asn Lys Asp Gly Glu
            420                 425                 430

Trp Val Leu Ala Pro Pro Glu Glu Asp Pro Thr Pro Glu Phe Asp
        435                 440                 445
```

<210> 3
<211> 10
<212> PRT
<213> Lycopersicon esculentum

<400> 3
```
Ile Thr Phe Thr Gly His Ser Leu Gly Ala
  1               5                  10
```

<210> 4
<211> 10
<212> PRT
<213> Dianthus caryophyllus

<400> 4
```
Ile Thr Phe Ala Gly His Ser Leu Gly Ala
  1               5                  10
```

<210> 5
<211> 19
<212> DNA
<213> Dianthus caryophyllus

<400> 5
acctactagg ttccgcgtc 19

<210> 6
<211> 923
<212> DNA
<213> Lycopersicon esculentum

<220>
<221> CDS
<222> (join(6..513, 845..921))

<400> 6

```
ctcta gac tat gag tgg gtg gat gtt tta ggt gct cgt cct gat tca          47
      Asp Tyr Glu Trp Val Asp Val Leu Gly Ala Arg Pro Asp Ser
        1               5                  10

gct gac tct ctt ctt cat cct aaa tct ctc caa aaa ggc att aac aac         95
Ala Asp Ser Leu Leu His Pro Lys Ser Leu Gln Lys Gly Ile Asn Asn
 15                  20                  25                  30

aag aac gat gag gat gag gac gag gac gag gat gag atc aaa gta atg        143
Lys Asn Asp Glu Asp Glu Asp Glu Asp Glu Asp Glu Ile Lys Val Met
                 35                  40                  45

gat ggg tgg ctt aag atc tac gtc tca agt aac ccg aag tcg tct ttc        191
Asp Gly Trp Leu Lys Ile Tyr Val Ser Ser Asn Pro Lys Ser Ser Phe
             50                  55                  60

acg aga cta agt gca aga gaa caa ctt caa gca aag att gaa aag tta        239
Thr Arg Leu Ser Ala Arg Glu Gln Leu Gln Ala Lys Ile Glu Lys Leu
         65                  70                  75

aga aat gag tat aaa gat gag aat ttg agc ata act ttt aca ggg cat        287
Arg Asn Glu Tyr Lys Asp Glu Asn Leu Ser Ile Thr Phe Thr Gly His
     80                  85                  90

agt ctt ggt gct agc tta gct gtt tta gct tca ttt gat gtg gtt gaa        335
Ser Leu Gly Ala Ser Leu Ala Val Leu Ala Ser Phe Asp Val Val Glu
 95                  100                 105                 110

aat ggt gtg cca gtt gat att cca gta tct gca att gta ttt ggt agt        383
Asn Gly Val Pro Val Asp Ile Pro Val Ser Ala Ile Val Phe Gly Ser
                115                 120                 125

cca caa gtt ggg aat aag gca ttc aat gaa aga atc aag aaa ttc tca        431
Pro Gln Val Gly Asn Lys Ala Phe Asn Glu Arg Ile Lys Lys Phe Ser
            130                 135                 140

aac ttg aat atc tta cat gtt aag aac aag att gat ctc att acc ctt        479
Asn Leu Asn Ile Leu His Val Lys Asn Lys Ile Asp Leu Ile Thr Leu
        145                 150                 155

tac cca agt gct ctg ttt ggg tat gtg aat tca g gtattgaagg               523
Tyr Pro Ser Ala Leu Phe Gly Tyr Val Asn Ser
    160                 165

aaaagatcat tacaattttg agctagattt ctcatatcgt cacactcaac taacagttat      583
```

tatatgagaa agtcactttc tttgtgaaaa aattgaatca acttttggaa ataatagtag 643

ttgagtgacc atatgagaaa tcaacactct actaacttta tgctataaga gaataggtta 703

aggtccatat gtttatactg tctgttcaat tagaatcata aaagtattac tagttaaatt 763

tgactacaat cttatgtaga catgaataaa ataaatccta cataaataag atttcctaca 823

actttaatga ttcttcaaca g gt ata gag cta gtc atc gat agc aga aag 873
Gly Ile Glu Leu Val Ile Asp Ser Arg Lys
170 175

tct ccg agt tta aag gat tca aaa gac atg ggc gac tgg cac aac ctc 921
Ser Pro Ser Leu Lys Asp Ser Lys Asp Met Gly Asp Trp His Asn Leu
180 185 190 195

ca 923

<210> 7
<211> 19
<212> DNA
<213> Artificial Sequence

<220>
<223> Description of Artificial Sequence: Primer

<400> 7
ctctagacta tgagtgggt 19

<210> 8
<211> 18
<212> DNA
<213> Artificial Sequence

<220>
<223> Description of Artificial Sequence: Primer

<400> 8
cgactggcac aacctcca 18

<210> 9
<211> 10
<212> PRT
<213> Arabidopsis sp.

<400> 9
Ile Thr Thr Cys Gly His Ser Leu Gly Ala
1 5 10

<210> 10
<211> 10
<212> PRT
<213> Ipomoea nil

<400> 10
Ile Thr Val Thr Gly His Ser Leu Gly Ser
1 5 10

<210> 11
<211> 447
<212> PRT
<213> Dianthus caryophyllus

<400> 11
Met Ala Ala Glu Ala Gln Pro Leu Gly Leu Ser Lys Pro Gly Pro Thr
1 5 10 15

Trp Pro Glu Leu Leu Gly Ser Asn Ala Trp Ala Gly Leu Leu Asn Pro
20 25 30

Leu Asn Asp Glu Leu Arg Glu Leu Leu Leu Arg Cys Gly Asp Phe Cys
35 40 45

Gln Val Thr Tyr Asp Thr Phe Ile Asn Asp Gln Asn Ser Ser Tyr Cys
50 55 60

Gly Ser Ser Arg Tyr Gly Lys Ala Asp Leu Leu His Lys Thr Ala Phe
65 70 75 80

Pro Gly Gly Ala Asp Arg Phe Asp Val Val Ala Tyr Leu Tyr Ala Thr
85 90 95

Ala Lys Val Ser Val Pro Glu Ala Phe Leu Leu Lys Ser Arg Ser Arg
100 105 110

Glu Lys Trp Asp Arg Glu Ser Asn Trp Ile Gly Tyr Val Val Val Ser
115 120 125

Asn Asp Glu Thr Ser Arg Val Ala Gly Arg Arg Glu Val Tyr Val Val
130 135 140

Trp Arg Gly Thr Cys Arg Asp Tyr Glu Trp Val Asp Val Leu Gly Ala
145 150 155 160

Gln Leu Glu Ser Ala His Pro Leu Leu Arg Thr Gln Gln Thr Thr His
165 170 175

Val Glu Lys Val Glu Asn Glu Glu Lys Lys Ser Ile His Lys Ser Ser
180 185 190

Trp Tyr Asp Cys Phe Asn Ile Asn Leu Leu Gly Ser Ala Ser Lys Asp
195 200 205

Lys Gly Lys Gly Ser Asp Asp Asp Asp Asp Asp Asp Pro Lys Val Met
210 215 220

Gln Gly Trp Met Thr Ile Tyr Thr Ser Glu Asp Pro Lys Ser Pro Phe
225 230 235 240

Thr Lys Leu Ser Ala Arg Thr Gln Leu Gln Thr Lys Leu Lys Cys Leu
245 250 255

```
Met Thr Lys Tyr Lys Asp Glu Thr Leu Ser Ile Thr Phe Ala Gly His
            260                 265                 270

Ser Leu Gly Ala Thr Leu Ser Val Val Ser Ala Phe Asp Ile Val Glu
        275                 280                 285

Asn Leu Thr Thr Glu Ile Pro Val Thr Ala Val Val Phe Gly Cys Pro
    290                 295                 300

Lys Val Gly Asn Lys Lys Phe Gln Gln Leu Phe Asp Ser Tyr Pro Asn
305                 310                 315                 320

Leu Asn Val Leu His Val Arg Asn Val Ile Asp Leu Ile Pro Leu Tyr
                325                 330                 335

Pro Val Lys Leu Met Gly Tyr Val Asn Ile Gly Ile Glu Leu Glu Ile
            340                 345                 350

Asp Ser Arg Lys Ser Thr Phe Leu Lys Asp Ser Lys Asn Pro Ser Asp
        355                 360                 365

Trp His Asn Leu Gln Ala Ile Leu His Val Val Ser Gly Trp His Gly
    370                 375                 380

Val Lys Gly Glu Phe Lys Val Val Asn Lys Arg Ser Val Ala Leu Val
385                 390                 395                 400

Asn Lys Ser Cys Asp Phe Leu Lys Glu Glu Cys Leu Val Pro Pro Ala
                405                 410                 415

Trp Trp Val Val Gln Asn Lys Gly Met Val Leu Asn Lys Asp Gly Glu
            420                 425                 430

Trp Val Leu Ala Pro Pro Glu Glu Asp Pro Thr Pro Glu Phe Asp
        435                 440                 445
```

<210> 12
<211> 418
<212> PRT
<213> Arabidopsis thaliana

<400> 12

```
Met Lys Arg Lys Lys Lys Glu Glu Glu Glu Glu Lys Leu Ile Val Thr
  1               5                  10                  15

Arg Glu Phe Ala Lys Arg Trp Arg Asp Leu Ser Gly Gln Asn His Trp
             20                  25                  30

Lys Gly Met Leu Gln Pro Leu Asp Gln Asp Leu Arg Glu Tyr Ile Ile
         35                  40                  45

His Tyr Gly Glu Met Ala Gln Ala Gly Tyr Asp Thr Phe Asn Ile Asn
     50                  55                  60

Thr Glu Ser Gln Phe Ala Gly Ala Ser Ile Tyr Ser Arg Lys Asp Phe
 65                  70                  75                  80
```

```
Phe Ala Lys Val Gly Leu Glu Ile Ala His Pro Tyr Thr Lys Tyr Lys
                85                  90                  95

Val Thr Lys Phe Ile Tyr Ala Thr Ser Asp Ile His Val Pro Glu Ser
            100                 105                 110

Phe Leu Leu Phe Pro Ile Ser Arg Glu Gly Trp Ser Lys Glu Ser Asn
        115                 120                 125

Trp Met Gly Tyr Val Ala Val Thr Asp Asp Gln Gly Thr Ala Leu Leu
    130                 135                 140

Gly Arg Arg Asp Ile Val Val Ser Trp Arg Gly Ser Val Gln Pro Leu
145                 150                 155                 160

Glu Trp Val Glu Asp Phe Glu Phe Gly Leu Val Asn Ala Ile Lys Ile
                165                 170                 175

Phe Gly Glu Arg Asn Asp Gln Val Gln Ile His Gln Gly Trp Tyr Ser
            180                 185                 190

Ile Tyr Met Ser Gln Asp Glu Arg Ser Pro Phe Thr Lys Thr Asn Ala
        195                 200                 205

Arg Asp Gln Val Leu Arg Glu Val Gly Arg Leu Leu Glu Lys Tyr Lys
    210                 215                 220

Asp Glu Glu Val Ser Ile Thr Ile Cys Gly His Ser Leu Gly Ala Ala
225                 230                 235                 240

Leu Ala Thr Asp Ser Ala Ile Asp Ile Val Ala Asn Gly Tyr Asn Arg
                245                 250                 255

Pro Lys Ser Arg Pro Asp Lys Ser Cys Pro Val Thr Ala Phe Val Phe
            260                 265                 270

Ala Ser Pro Arg Val Gly Asp Ser Asp Phe Arg Lys Leu Phe Ser Gly
        275                 280                 285

Leu Glu Asp Ile Arg Val Leu Arg Thr Arg Asn Leu Phe Asp Val Ile
    290                 295                 300

Pro Ile Tyr Pro Pro Ile Gly Tyr Ser Glu Val Gly Asp Glu Phe Pro
305                 310                 315                 320

Ile Asp Thr Arg Lys Ser Pro Tyr Met Lys Ser Pro Gly Asn Leu Ala
                325                 330                 335

Thr Phe His Cys Leu Glu Gly Tyr Leu His Gly Val Ala Gly Thr Gln
            340                 345                 350

Gly Thr Asn Lys Ala Asp Leu Phe Arg Leu Asp Val Glu Arg Ala Ile
        355                 360                 365

Gly Leu Val Asn Lys Ser Val Asp Gly Leu Lys Asp Glu Cys Met Val
    370                 375                 380
```

```
Pro Gly Lys Trp Arg Val Leu Lys Asn Lys Gly Ala Gln Gln Asp Asp
385                 390                 395                 400

Gly Ser Trp Glu Leu Val Asp His Glu Ile Asp Asp Asn Glu Asp Leu
                405                 410                 415

Asp Phe
```

<210> 13
<211> 401
<212> PRT
<213> Ipomoea sp.

<400> 13

```
Met Ser Gly Ile Ala Lys Arg Trp Lys Val Leu Ser Gly Ser Asp Asn
  1               5                  10                  15

Trp Glu Gly Leu Leu Glu Pro Leu Asp Ser Asp Leu Arg Arg Tyr Leu
             20                  25                  30

Ile His Tyr Gly Thr Met Val Ser Pro Ala Thr Asp Ser Phe Ile Asn
         35                  40                  45

Glu Ala Ala Ser Lys Asn Val Gly Leu Pro Arg Tyr Ala Arg Arg Asn
     50                  55                  60

Leu Leu Ala Asn Cys Gly Leu Val Lys Gly Asn Pro Phe Lys Tyr Glu
 65                  70                  75                  80

Val Thr Lys Tyr Phe Tyr Ala Pro Ser Thr Ile Pro Leu Pro Asp Glu
                 85                  90                  95

Gly Tyr Asn Val Arg Ala Thr Arg Ala Asp Ala Val Leu Lys Glu Ser
            100                 105                 110

Asn Trp Asn Gly Tyr Val Ala Val Ala Thr Asp Glu Gly Lys Val Ala
        115                 120                 125

Leu Gly Arg Arg Asp Ile Leu Ile Val Trp Arg Gly Thr Ile Arg Lys
    130                 135                 140

Ser Glu Trp Asn Glu Asn Leu Thr Phe Trp Phe Val Lys Ala Pro Leu
145                 150                 155                 160

Phe Phe Gly Gln Asn Ser Asp Pro Leu Val His Lys Gly Trp Tyr Asp
                165                 170                 175

Met Tyr Thr Thr Ile Asn Gln Asp Ser Gln Leu Asn Glu Lys Ser Ala
            180                 185                 190

Arg Asp Gln Ile Arg Glu Glu Val Ala Arg Leu Val Glu Leu Tyr Lys
        195                 200                 205

Asp Glu Asp Ile Ser Ile Thr Val Thr Gly His Ser Leu Gly Ser Ser
    210                 215                 220
```

```
Met Ala Thr Leu Asn Ala Val Asp Leu Ala Ala Asn Pro Ile Asn Asn
225                 230                 235                 240

Asn Lys Asn Ile Leu Val Thr Ala Phe Leu Tyr Ala Ser Pro Lys Val
                245                 250                 255

Gly Asp Glu Asn Phe Lys Asn Val Ile Ser Asn Gln Gln Asn Leu Arg
            260                 265                 270

Ala Leu Arg Ile Ser Asp Val Asn Asp Ile Val Thr Ala Val Pro Pro
        275                 280                 285

Phe Gly Trp Lys Glu Cys Asp Asn Thr Ala Ile Leu Tyr Gly Asp Val
    290                 295                 300

Gly Val Gly Leu Val Ile Asp Ser Lys Lys Ser His Tyr Leu Lys Pro
305                 310                 315                 320

Asp Phe Pro Asn Leu Ser Thr His Asp Leu Met Leu Tyr Met His Ala
                325                 330                 335

Ile Asp Gly Tyr Gln Gly Ser Gln Gly Gly Phe Glu Arg Gln Glu Asp
            340                 345                 350

Phe Asp Leu Ala Lys Val Asn Lys Tyr Gly Asp Tyr Leu Lys Ala Glu
        355                 360                 365

Tyr Pro Ile Pro Ile Gly Trp Phe Asn Ile Lys Asp Lys Gly Met Gln
    370                 375                 380

Gln Asp Asp Gly Asn Tyr Ile Leu Asp Asp His Glu Val Asp Lys Thr
385                 390                 395                 400

Phe
```

<210> 14
<211> 448
<212> PRT
<213> Arabidopsis thaliana

<400> 14

```
Met Thr Ala Glu Asp Ile Arg Arg Arg Asp Lys Lys Thr Glu Glu Glu
  1               5                  10                  15

Arg Arg Leu Arg Asp Thr Trp Arg Lys Ile Gln Gly Glu Asp Asp Trp
             20                  25                  30

Ala Gly Leu Met Asp Pro Met Asp Pro Ile Leu Arg Ser Glu Leu Ile
         35                  40                  45

Arg Tyr Gly Glu Met Ala Gln Ala Cys Tyr Asp Ala Phe Asp Phe Asp
     50                  55                  60

Pro Ala Ser Lys Tyr Cys Gly Thr Ser Arg Phe Thr Arg Leu Glu Phe
 65                  70                  75                  80
```

```
Phe Asp Ser Leu Gly Met Ile Asp Ser Gly Tyr Glu Val Ala Arg Tyr
                 85                  90                  95

Leu Tyr Ala Thr Ser Asn Ile Asn Leu Pro Asn Phe Phe Ser Lys Ser
            100                 105                 110

Arg Trp Ser Lys Val Trp Ser Lys Asn Ala Asn Trp Met Gly Tyr Val
        115                 120                 125

Ala Val Ser Asp Asp Glu Thr Ser Arg Asn Arg Leu Gly Arg Arg Asp
    130                 135                 140

Ile Ala Ile Ala Trp Arg Gly Thr Val Thr Lys Leu Glu Trp Ile Ala
145                 150                 155                 160

Asp Leu Lys Asp Tyr Leu Lys Pro Val Thr Glu Asn Lys Ile Arg Cys
                165                 170                 175

Pro Asp Pro Ala Val Lys Val Glu Ser Gly Phe Leu Asp Leu Tyr Thr
            180                 185                 190

Asp Lys Asp Thr Thr Cys Lys Phe Ala Arg Phe Ser Ala Arg Glu Gln
        195                 200                 205

Ile Leu Thr Glu Val Lys Arg Leu Val Glu Glu His Gly Asp Asp Asp
    210                 215                 220

Asp Ser Asp Leu Ser Ile Thr Val Thr Gly His Ser Leu Gly Gly Ala
225                 230                 235                 240

Leu Ala Ile Leu Ser Ala Tyr Asp Ile Ala Glu Met Arg Leu Asn Arg
                245                 250                 255

Ser Lys Lys Gly Lys Val Ile Pro Val Thr Ala Val Leu Thr Tyr Gly
            260                 265                 270

Gly Pro Arg Val Gly Asn Val Arg Phe Arg Glu Arg Met Glu Glu Leu
        275                 280                 285

Gly Val Lys Val Met Arg Val Val Asn Val His Asp Val Val Pro Lys
    290                 295                 300

Ser Pro Gly Leu Phe Leu Asn Glu Ser Arg Pro His Ala Leu Met Lys
305                 310                 315                 320

Ile Ala Glu Gly Leu Pro Trp Cys Tyr Ser His Val Gly Glu Glu Leu
                325                 330                 335

Ala Leu Asp His Gln Asn Ser Pro Phe Leu Lys Pro Ser Val Asp Val
            340                 345                 350

Ser Thr Ala His Asn Leu Glu Ala Met Leu His Leu Leu Asp Gly Tyr
        355                 360                 365

His Gly Lys Gly Glu Arg Phe Val Leu Ser Ser Gly Arg Asp His Ala
    370                 375                 380
```

```
Leu Val Asn Lys Ala Ser Asp Phe Leu Lys Glu His Leu Gln Ile Pro
385                 390                 395                 400

Pro Phe Trp Arg Gln Asp Ala Asn Lys Gly Met Val Arg Asn Ser Glu
                405                 410                 415

Gly Arg Trp Ile Gln Ala Glu Arg Leu Arg Phe Glu Asp His His Ser
            420                 425                 430

Pro Asp Ile His His His Leu Ser Gln Leu Arg Leu Asp His Pro Cys
        435                 440                 445
```

<210> 15
<211> 1167
<212> DNA
<213> Arabidopsis sp.

<220>
<221> CDS
<222> (1)..(1044)

<400> 15

```
cgg gtc gac cca cgc gtc cgc gaa aac gct tcc gac tac gag gtt gta   48
Arg Val Asp Pro Arg Val Arg Glu Asn Ala Ser Asp Tyr Glu Val Val
  1               5                  10                  15

aac ttc ctc tac gcc aca gct cgt gtt tct ctc ccc gaa ggt ttg ctt   96
Asn Phe Leu Tyr Ala Thr Ala Arg Val Ser Leu Pro Glu Gly Leu Leu
             20                  25                  30

ctc caa tca caa tca aga gat tct tgg gac cgt gag tct aac tgg ttt   144
Leu Gln Ser Gln Ser Arg Asp Ser Trp Asp Arg Glu Ser Asn Trp Phe
         35                  40                  45

ggc tac att gct gtc acg tct gat gaa cgg tct aag gct tta gga cgc   192
Gly Tyr Ile Ala Val Thr Ser Asp Glu Arg Ser Lys Ala Leu Gly Arg
     50                  55                  60

cgt gag atc tat ata gct ttg aga gga acg agc agg aac tat gag tgg   240
Arg Glu Ile Tyr Ile Ala Leu Arg Gly Thr Ser Arg Asn Tyr Glu Trp
 65                  70                  75                  80

gtc aat gtt ttg ggt gct agg cca act tca gct gac ccc ttg ctg cac   288
Val Asn Val Leu Gly Ala Arg Pro Thr Ser Ala Asp Pro Leu Leu His
                85                  90                  95

gga ccc gag cag gat ggt tct ggt ggt gta gtt gaa ggt acg act ttt   336
Gly Pro Glu Gln Asp Gly Ser Gly Gly Val Val Glu Gly Thr Thr Phe
            100                 105                 110

gat agt gac agt gaa gat gaa gaa ggg tgt aag gtg atg ctc ggg tgg   384
Asp Ser Asp Ser Glu Asp Glu Glu Gly Cys Lys Val Met Leu Gly Trp
        115                 120                 125

ctc aca atc tat act tct aat cac ccc gaa tcg aaa ttc act aag ctg   432
Leu Thr Ile Tyr Thr Ser Asn His Pro Glu Ser Lys Phe Thr Lys Leu
    130                 135                 140
```

```
agt cta cgg tca cag ttg tta gcc aag atc aag gag ctt ctg ttg aag   480
Ser Leu Arg Ser Gln Leu Leu Ala Lys Ile Lys Glu Leu Leu Leu Lys
145                 150                 155                 160

tat aag gac gag aaa ccg agc att gtg ttg act gga cat agc ttg gga   528
Tyr Lys Asp Glu Lys Pro Ser Ile Val Leu Thr Gly His Ser Leu Gly
                165                 170                 175

cct aca gag gct gtt ctg gcc gcc tat gat ata gct gag aac ggt tcc   576
Pro Thr Glu Ala Val Leu Ala Ala Tyr Asp Ile Ala Glu Asn Gly Ser
            180                 185                 190

agt gat gat gtt ccg gtc act gct ata gtc ttt ggt tgt cca cag gta   624
Ser Asp Asp Val Pro Val Thr Ala Ile Val Phe Gly Cys Pro Gln Val
        195                 200                 205

gga aac aag gag ttc aga gac gaa gta atg agt cac aag aac tta aag   672
Gly Asn Lys Glu Phe Arg Asp Glu Val Met Ser His Lys Asn Leu Lys
    210                 215                 220

atc ctc cat gta agg aac acg att gat ctc tta act cga tac cca ggg   720
Ile Leu His Val Arg Asn Thr Ile Asp Leu Leu Thr Arg Tyr Pro Gly
225                 230                 235                 240

gga ctt tta ggg tat gtg gac ata gga ata aac ttt gtg atc gat aca   768
Gly Leu Leu Gly Tyr Val Asp Ile Gly Ile Asn Phe Val Ile Asp Thr
                245                 250                 255

aag aag tca ccg ttc cta agc gat tca agg aat cca ggg gat tgg cat   816
Lys Lys Ser Pro Phe Leu Ser Asp Ser Arg Asn Pro Gly Asp Trp His
            260                 265                 270

aat ctt cag gcg atg tta cat gtt gta gct gga tgg aat ggg aag aaa   864
Asn Leu Gln Ala Met Leu His Val Val Ala Gly Trp Asn Gly Lys Lys
        275                 280                 285

gga gag ttt aaa ctg atg gtt aag aga agt att gca tta gtg aac aag   912
Gly Glu Phe Lys Leu Met Val Lys Arg Ser Ile Ala Leu Val Asn Lys
    290                 295                 300

tca tgc gag ttc ttg aaa gct gag tgt ttg gtg cca gga tct tgg tgg   960
Ser Cys Glu Phe Leu Lys Ala Glu Cys Leu Val Pro Gly Ser Trp Trp
305                 310                 315                 320

gta gag aag aac aaa gga ctg atc aag aac gaa gat ggt gaa tgg gtt   1008
Val Glu Lys Asn Lys Gly Leu Ile Lys Asn Glu Asp Gly Glu Trp Val
                325                 330                 335

ctt gct ccc gtt gaa gaa gaa cct gta cct gaa ttc taaattgtat        1054
Leu Ala Pro Val Glu Glu Glu Pro Val Pro Glu Phe
            340                 345

ttctgtattt ttctctaagg tcatgataaa tcaacaataa gcagttcaac tatgtgatga 1114

aaagacccaa gttattatat tgatatgagt ttatgagata aaaaaaaaaa aaa        1167
```

<210> 16
<211> 348
<212> PRT
<213> Arabidopsis sp.

<400> 16

```
Arg Val Asp Pro Arg Val Arg Glu Asn Ala Ser Asp Tyr Glu Val Val
  1               5                  10                  15

Asn Phe Leu Tyr Ala Thr Ala Arg Val Ser Leu Pro Glu Gly Leu Leu
             20                  25                  30

Leu Gln Ser Gln Ser Arg Asp Ser Trp Asp Arg Glu Ser Asn Trp Phe
         35                  40                  45

Gly Tyr Ile Ala Val Thr Ser Asp Glu Arg Ser Lys Ala Leu Gly Arg
     50                  55                  60

Arg Glu Ile Tyr Ile Ala Leu Arg Gly Thr Ser Arg Asn Tyr Glu Trp
 65                  70                  75                  80

Val Asn Val Leu Gly Ala Arg Pro Thr Ser Ala Asp Pro Leu Leu His
                 85                  90                  95

Gly Pro Glu Gln Asp Gly Ser Gly Gly Val Val Glu Gly Thr Thr Phe
            100                 105                 110

Asp Ser Asp Ser Glu Asp Glu Glu Gly Cys Lys Val Met Leu Gly Trp
        115                 120                 125

Leu Thr Ile Tyr Thr Ser Asn His Pro Glu Ser Lys Phe Thr Lys Leu
    130                 135                 140

Ser Leu Arg Ser Gln Leu Leu Ala Lys Ile Lys Glu Leu Leu Leu Lys
145                 150                 155                 160

Tyr Lys Asp Glu Lys Pro Ser Ile Val Leu Thr Gly His Ser Leu Gly
                165                 170                 175

Pro Thr Glu Ala Val Leu Ala Ala Tyr Asp Ile Ala Glu Asn Gly Ser
            180                 185                 190

Ser Asp Asp Val Pro Val Thr Ala Ile Val Phe Gly Cys Pro Gln Val
        195                 200                 205

Gly Asn Lys Glu Phe Arg Asp Glu Val Met Ser His Lys Asn Leu Lys
    210                 215                 220

Ile Leu His Val Arg Asn Thr Ile Asp Leu Leu Thr Arg Tyr Pro Gly
225                 230                 235                 240

Gly Leu Leu Gly Tyr Val Asp Ile Gly Ile Asn Phe Val Ile Asp Thr
                245                 250                 255

Lys Lys Ser Pro Phe Leu Ser Asp Ser Arg Asn Pro Gly Asp Trp His
            260                 265                 270
```

```
Asn Leu Gln Ala Met Leu His Val Val Ala Gly Trp Asn Gly Lys Lys
        275                 280                 285

Gly Glu Phe Lys Leu Met Val Lys Arg Ser Ile Ala Leu Val Asn Lys
    290                 295                 300

Ser Cys Glu Phe Leu Lys Ala Glu Cys Leu Val Pro Gly Ser Trp Trp
305                 310                 315                 320

Val Glu Lys Asn Lys Gly Leu Ile Lys Asn Glu Asp Gly Glu Trp Val
                325                 330                 335

Leu Ala Pro Val Glu Glu Glu Pro Val Pro Glu Phe
            340                 345
```

<210> 17
<211> 195
<212> PRT
<213> Lycopersicon esculentum

<400> 17

```
Asp Tyr Glu Trp Val Asp Val Leu Gly Ala Arg Pro Asp Ser Ala Asp
  1               5                  10                  15

Ser Leu Leu His Pro Lys Ser Leu Gln Lys Gly Ile Asn Asn Lys Asn
             20                  25                  30

Asp Glu Asp Glu Asp Glu Asp Glu Asp Glu Ile Lys Val Met Asp Gly
         35                  40                  45

Trp Leu Lys Ile Tyr Val Ser Ser Asn Pro Lys Ser Ser Phe Thr Arg
     50                  55                  60

Leu Ser Ala Arg Glu Gln Leu Gln Ala Lys Ile Glu Lys Leu Arg Asn
 65                  70                  75                  80

Glu Tyr Lys Asp Glu Asn Leu Ser Ile Thr Phe Thr Gly His Ser Leu
                 85                  90                  95

Gly Ala Ser Leu Ala Val Leu Ala Ser Phe Asp Val Val Glu Asn Gly
            100                 105                 110

Val Pro Val Asp Ile Pro Val Ser Ala Ile Val Phe Gly Ser Pro Gln
        115                 120                 125

Val Gly Asn Lys Ala Phe Asn Glu Arg Ile Lys Lys Phe Ser Asn Leu
    130                 135                 140

Asn Ile Leu His Val Lys Asn Lys Ile Asp Leu Ile Thr Leu Tyr Pro
145                 150                 155                 160

Ser Ala Leu Phe Gly Tyr Val Asn Ser Gly Ile Glu Leu Val Ile Asp
                165                 170                 175

Ser Arg Lys Ser Pro Ser Leu Lys Asp Ser Lys Asp Met Gly Asp Trp
            180                 185                 190
```

His Asn Leu
195

INTERNATIONAL SEARCH REPORT

Inter onal Application No
PCT/US 00/03494

A. CLASSIFICATION OF SUBJECT MATTER
IPC 7 C12N15/82 C12N15/55 C12N9/18 C12N5/10 A01H5/00

According to International Patent Classification (IPC) or to both national classification and IPC

B. FIELDS SEARCHED

Minimum documentation searched (classification system followed by classification symbols)
IPC 7 C12N A01H

Documentation searched other than minimum documentation to the extent that such documents are included in the fields searched

Electronic data base consulted during the international search (name of data base and, where practical, search terms used)
EPO-Internal, WPI Data, PAJ, STRAND, BIOSIS

C. DOCUMENTS CONSIDERED TO BE RELEVANT

Category *	Citation of document, with indication, where appropriate, of the relevant passages	Relevant to claim No.
Y	HONG, Y., ET AL.: "a cDNA clone from Dianthus caryophyllus encoding a lipid-protein-particle associated lipase" EMBL SEQUENCE DATA LIBRARY, 6 January 1999 (1999-01-06), XP002143322 heidelberg, germany accession no.AF026480 -/-	1-5

[X] Further documents are listed in the continuation of box C.

[X] Patent family members are listed in annex.

* Special categories of cited documents :

"A" document defining the general state of the art which is not considered to be of particular relevance

"E" earlier document but published on or after the international filing date

"L" document which may throw doubts on priority claim(s) or which is cited to establish the publication date of another citation or other special reason (as specified)

"O" document referring to an oral disclosure, use, exhibition or other means

"P" document published prior to the international filing date but later than the priority date claimed

"T" later document published after the international filing date or priority date and not in conflict with the application but cited to understand the principle or theory underlying the invention

"X" document of particular relevance; the claimed invention cannot be considered novel or cannot be considered to involve an inventive step when the document is taken alone

"Y" document of particular relevance; the claimed invention cannot be considered to involve an inventive step when the document is combined with one or more other such documents, such combination being obvious to a person skilled in the art.

"&" document member of the same patent family

Date of the actual completion of the international search	Date of mailing of the international search report
25 July 2000	09/08/2000

Name and mailing address of the ISA	Authorized officer
European Patent Office, P.B. 5818 Patentlaan 2 NL - 2280 HV Rijswijk Tel. (+31-70) 340-2040, Tx. 31 651 epo nl, Fax: (+31-70) 340-3016	Holtorf, S

Form PCT/ISA/210 (second sheet) (July 1992)

INTERNATIONAL SEARCH REPORT

Inter onal Application No
PCT/US 00/03494

C.(Continuation) DOCUMENTS CONSIDERED TO BE RELEVANT

Category *	Citation of document, with indication, where appropriate, of the relevant passages	Relevant to claim No.
Y	BURGER L ET AL: "RELATIONSHIP BETWEEN CHANGES IN MEMBRANE PERMEABILITY RESPIRATION RATE ACTIVITIES OF LIPASE AND PHOSPHOLIPASE C AND ULTRASTRUCTURE IN SENESCING PETALS OF DIANTHUS-CARYOPHYLLUS CULTIVAR WHITE-SIM" SOUTH AFRICAN JOURNAL OF BOTANY, vol. 52, no. 3, 1986, pages 195-200, XP000925543 ISSN: 0254-6299 the whole document	1-5
Y	BROWN J H ET AL: "MOLECULAR SPECIES SPECIFICITY OF PHOSPHOLIPID BREAKDOWN IN MICROSOMAL MEMBRANES OF SENESCING CARNATION FLOWERS" PLANT PHYSIOLOGY (BETHESDA), vol. 85, no. 3, 1987, pages 679-683, XP000925613 ISSN: 0032-0889 the whole document	1-5
A	WO 97 13851 A (HANDA AVTAR K ;KAUSCH KURT D (US); PURDUE RESEARCH FOUNDATION (US)) 17 April 1997 (1997-04-17) the whole document	
A	WO 96 35792 A (ALLRAD NO 1 PTY LTD ;FLORIGENE INVESTMENTS PTY LTD (AU); MICHAEL M) 14 November 1996 (1996-11-14) the whole document	
A	HUANG A: "Plant Lipases" PLANT LIPASES,NL,ELSEVIER, AMSTERDAM, 1984, pages 419-442, XP002112793 the whole document	
A	WO 95 07993 A (ZENECA LTD ;SMART CATHERINE MARGARET (GB); THOMAS HOWARD (GB); HOS) 23 March 1995 (1995-03-23) the whole document	
T	HONG,Y., ET AL. : "an ethylene-induced cDNa encoding a lipase expressed atthe onset of senescence" PROCEEDINGS OF THE NATIONAL ACADEMY OF SCIENCES OF THE USA, vol. 97, no. 15, 18 July 2000 (2000-07-18), XP002143325 the whole document	

1

FURTHER INFORMATION CONTINUED FROM PCT/ISA/ 210

Continuation of Box I.2

The SEQID 4 mentioned in claim 3 does not represent a nucleotide sequence but an amino acid sequence. Claim 3 was searched as if referring to a DNA molecule that contains the nucleotide sequence of SEQID 1.

The applicant's attention is drawn to the fact that claims, or parts of claims, relating to inventions in respect of which no international search report has been established need not be the subject of an international preliminary examination (Rule 66.1(e) PCT). The applicant is advised that the EPO policy when acting as an International Preliminary Examining Authority is normally not to carry out a preliminary examination on matter which has not been searched. This is the case irrespective of whether or not the claims are amended following receipt of the search report or during any Chapter II procedure.

INTERNATIONAL SEARCH REPORT

formation on patent family members

Inter 'onal Application No
PCT/US 00/03494

Patent document cited in search report	Publication date	Patent family member(s)	Publication date
WO 9713851 A	17-04-1997	AU 7443596 A	30-04-1997
		CA 2234107 A	17-04-1997
		EP 0859836 A	26-08-1998
WO 9635792 A	14-11-1996	AU 703841 B	01-04-1999
		AU 5493096 A	29-11-1996
		EP 0824591 A	25-02-1998
		JP 11504815 T	11-05-1999
WO 9507993 A	23-03-1995	AU 696417 B	10-09-1998
		AU 7619494 A	03-04-1995
		CA 2172842 A	23-03-1995
		EP 0719341 A	03-07-1996